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THE CERCARICIDAL ACTION OF NORMAL SERUMS[1]

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It is well known that the normal blood serum of various animals possesses the power to destroy many species of bacteria. The normal serum of man as well as of a few other Primates is known to have the additional capacity to lyse some species of trypanosomes and to neutralize certain filterable viruses. No such antagonistic property of the normal serum against multicellular forms has been described to our knowledge. During the past summer (1935) it was our privilege, in the Helminthology Laboratory of the University of Michigan Biological Station at Douglas Lake, Michigan, to test the normal serums of various animals upon a considerable number of species of cercariae, one of the larval stages of trematodes. A positive cercaricidal effect was obtained in many instances, as will be seen.

METHOD FOR DEMONSTRATING THE CERCARICIDAL ACTION

A serum can be tested and titrated for cercaricidal action in the following manner: Five-tenths cubic centimeter portions of successive dilutions of the serum in 0.75 per cent sodium chloride solution are pipetted into a series of small tubes. Control tubes, one with 0.5 cubic centimeter of the salt solution and one with 0.5 cubic centimeter of tap water, also are prepared. To each tube is then added 0.5 cubic centimeter of a uniform suspension of freshly-shed cercariae.[2] The cercariae in each tube

[1] From the Biological Station of the University of Michigan and the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York. It is a pleasure to acknowledge the favor of Dr. G. R. La Rue, Director of the University of Michigan Biology Station, for permission to use the facilities of the Station, and the many kindnesses of Dr. W. W. Cort, of The Johns Hopkins University, and of Dr. L. J. Thomas, of the University of Illinois, members of the staff of the Biology Station, in whose laboratories these studies were carried on. The several visiting investigators with whom we were privileged to associate were all generous in their assistance and advice.

[2] The cercariae suspensions were obtained by placing heavily infected snails collected in the Douglas Lake area in wide-mouth bottles (*e. g.*, half-pint milk bottles) half-full of tap water. Usually sufficient cercariae came from the snails to render unnecessary the concentration of the tap water suspension, since, after shaking to render the suspension uniform, from 25 to 100 organisms were present in a cubic centimeter of fluid. The cercariae were used generally within 12 hours of their emergence from the snails.

are counted by examining the tube under the low power binocular microscope. Generally, tests with from 25 to 75 cercariae per cubic centimeter of the fluid in the tube prove satisfactory. All tubes are then incubated for an hour at 37° C. After this interval, the living actively moving cercariae are again counted and the results recorded. The cercaricidal titer of the serum is taken as the highest dilution of the serum which kills all the cercariae. The results of the titration of the serums of man, rat, and duck against three species of cercariae are presented in Table 1.

For the work described in this paper, the method which we have described proved satisfactory. It is probable that observations made after greater intervals than one hour would reveal more delicate effects of the serum. However, the relatively brief natural life of the cercariae, particularly that of certain species, and dangers incident to the inevitable bacterial contamination of the cercariae suspension from the snail host must be considered in the preparation of protocols.

GENERAL CONSIDERATIONS

1. *Kinds of serums which manifest cercaricidal action.* In an effort to determine how widespread among vertebrate animals the cercaricidal property of serum should prove to be, we have tested serums of one or more representatives of each class of the vertebrate phylum. The serums of man, white rat, and cat were included for mammals, those of domesticated duck, and herring gull (*Larus argentatus smithsonianus*) for birds, that of water snake (*Natrix sipedon sipedon*) for reptiles, that of green frog (*Rana clamitans*) for amphibians, and that of bullhead (*Ameiurus nebulosus*) for fish. These were tested against each of a number of species of cercariae. It was found that all of the serums, save that of the cat, were active against at least some species of cercariae, all tests being carried out with a standard 1:6 final dilution of the fresh serum. The data upon these tests are shown in Table 2.

The failure of cat serum, alone among those tested, to exhibit cercaricidal action was studied somewhat further. Blood was drawn from four different cats—three young animals of from five to ten weeks old and one adult. The undiluted serum was added to an equal volume of the cercaria suspension. Yet we obtained no evidence that cat serum possesses activity against any species of cercaria, within the standard test interval of one hour at 37° C. The cat serum has, however, no anti-cercaricidal property, for it does not inhibit the cercaricidal power of an active serum with which it may be mixed. Proof of this fact is given in Table 3.

2. *Species of cercariae affected by serum.* Some species of cercariae were susceptible to a greater number of serums than others. For ex-

TABLE 1.—*Titration of the serum of man, white rat, and duck against three species of cercariae*

Species of cercaria	Serum dilution (Final)	Human serum			Rat serum			Duck serum			Control solutions		
		Tube No.	Number of living cercariae		Tube No.	Number of living cercariae		Tube No.	Number of living cercariae			Number of living cercariae	
			At start	After 1 hr. at 37° C.		At start	After 1 hr. at 37° C.		At start	After 1 hr. at 37° C.		At start	After 1 hr. at 37° C.
Cercariae physae	1– 12	1	30	0	8	31	0	15	30	0	0.75% NaCl	22	22
	1– 24	2	25	0	9	35	0	16	26	0			
	1– 48	3	24	0	10	28	0	17	28	27			
	1– 96	4	29	1	11	27	0	18	18	26	Tap water	36	32
	1–192	5	33	20	12	30	0	19	29	29			
	1–384	6	29	32	13	25	28	20	30	27			
	1–768	7	38	40	14	27	24	21	28	25			
Cercaria of *Schistosomatium douthitti*	1– 12	1	6	6	5	5	5	9	11	10	0.75% NaCl	6	6
	1– 24	2	5	5	6	6	4	10	7	7			
	1– 48	3	8	7	7	6	6	11	11	9	Tap water	8	8
	1– 96	4	11	10	8	4	5	12	6	7			
Cercaria Stagnicolae	1– 12	1	16	0	6	12	0	11	9	9	0.75% NaCl	12	11
	1– 24	2	10	0	7	11	0	12	12	10			
	1– 48	3	12	9	8	14	0	13	13	12			
	1– 96	4	12	12	9	10	10	14	12	10	Tap water	9	8
	1–192	5	9	10	10	10	9	15	9	8			

TABLE 2.—*The effect of the serums of various animals upon different species of cercariae*

Species of *Cercariae*	Group of *Cercaria*	Snail Host	Vertebrate serums							References to Descriptions of *Cercariae*
			Man	Rat	Cat	Herring Gull	Water Snake	Green Frog	Bullhead Fish	
Cercaria of *Schistosomatium douthitti*	Schistosome	*Lymnaea stagnalis*	o	o	o	o	+	+		Cort, 1914; Price, 1931
Cercaria stagnicolae	Schistosome	*Stagnicola emarginata angulata*	⊕	+	o	⊕	+	+		Talbot, in press
Cercaria physellae	Schistosome	*Physella parkeri*		+		⊕				Talbot, in press
Cercaria elephantis	Schistosome	*Helisoma trivolvis*	+	+	o	+	+			Cort, 1917
Cercaria bessiae	Strigeid	*Helisoma trivolvis*	+	+			+	+	+	Cort and Brooks, 1928
Cercaria of *Cotylurus communis*	Strigeid	*Stagnicola emarginata angulata*		o	o	o	⊕	+		Van Haitsma, 1930, 1931; La Rue, 1932
Cercaria of *Diplostomum flexicaudum*	Strigeid	*Stagnicola emarginata angulata*	o	⊕	o	⊕	⊕	+	⊕	Cort and Brooks, 1928
Cercaria of *Diplodiscus temperatus*	Amphistome	*Helisoma trivolvis*	+	+	o	+	+	+		Krull and Price, 1932
Cercaria of *Macroderoides typicus*	Stylet	*Helisoma trivolvis*		+					o	McMullen, 1935
Cercaria of *Eustomos chelydrae*	Stylet	*Lymnaea stagnalis*					+			McMullen, 1935

+ : all cercariae killed ; o : cercariae not killed ; ⊕ : some cercariae killed.
All tests made with serum in final dilution of 1 : 6.
We are grateful to Dr. W. W. Cort and others in his laboratory at the Biology Station for identifying the species of cercariae used in this work.

TABLE 3.—*Tests with cat serum, illustrating its lack of cercaricidal power, and its failure to inhibit an active serum with which it may be mixed*

Serum or Control Solution	Tube No.	No. of living cercariae (*C. physae*)*	
		At start	Afte 1 hr. at 37° C.
Rat serum (0.25 cc.) + tap water (0.25 cc.) ..	1	50	0
Rat serum (0.25 cc.) + cat serum (0.25 cc.) ..	2	50	0
Cat serum (0.5 cc.)	3	50	48
Saline (0.5 cc.)	4	50	45
Tap water (0.5 cc.)	5	50	50

* 0.5 cc. of suspension of *Cercaria physae* added to each tube.

ample, the cercariae of *Schistosomatium douthitti* were resistant to the serums of all the warm-blooded forms with which it was tested, but was killed by the serum of the water snake and the green frog. Another schistosome, *Cercaria elephantis,* was readily destroyed by the serum of man, rat, herring gull, and water snake, surviving only after contact with cat serum. Among the strigeids, *Cercaria physae* proved readily susceptible to all serums used, save that of the cat, but the cercariae of *Diplostomum flexicaudum* proved highly resistant to all serums, except that of the green frog. The amphistome cercariae of *Diplodiscus temperatus* were readily affected by all serums other than that of the cat. The results of tests for the susceptibility or resistance of the various species of cercariae to the serums of different vertebrates are presented in Table 2.

(a) *Differences in the susceptibility of a given species of cercaria coming from several snail hosts.* The freshly-shed cercariae (*Cercaria physae*) of four snails (*Physella gyrina*) were tested simultaneously against the fresh serum of a rat. A considerable difference in the susceptibility of the cercariae from the different snails was shown by the results, those forms from one snail being all killed in the standard test, and those from another being only slightly reduced in number. The results of the tests are included in Table 4. It is indicated that great care must be exercised in the selection of the cercariae used in comparative tests, even when these cercariae be of a single species.

(b) *Susceptibility of the cercariae at various ages after emerging from a given snail host.* Cercariae (*C. physae*) were tested for serum susceptibility at various intervals after emergence from a single snail specimen (*Physella gyrina*). Three ages or cercariae were used—one, from 0 to 4 hours old; another, from 4 to 8 hours old; and a third, from 8 to 13 hours old. In each case, all the cercariae were killed by fresh rat serum in a final dilution of 1 : 6. No evidence was obtained that the serum susceptibility of cercariae changes with age. The results of these tests are presented in Table 5.

TABLE 4.—*Tests illustrating the difference in susceptibility to serum of cercariae of a single species coming from different snail hosts, all of the same species**

Serum or control solution	Snail Host No. 1			Snail Host No. 2			Snail Host No. 3			Snail Host No. 4		
	Tube No.	Number of living cercariae		Tube No.	Number of living cercariae		Tube No.	Number of living cercariae		Tube No.	Number of living cercariae	
		At start	After 1 hr. at 37° C.		At start	After 1 hr. at 37° C.		At start	After 1 hr. at 37° C.		At start	After 1 hr. at 37° C.
Rat serum	1	24	6	4	33	28	7	23	0	10	25	1
Saline (0.75% NaCl) ..	2	23	25	5	38	35	8	26	22	11	25	24
Tap water	3	20	22	6	30	27	9	28	33	12	28	22

* Cercariae used : *Cercaria physae.*
Snail hosts : *Physella gyrina.*
Rat serum used in final dilution of 1 : 6.

TABLE 5.—*Tests upon the susecptibility to serum of cercariae of different age**

Age of cercariae in hours	Rat Serum			0.75% NaCl			Tap Water		
	Tube No.	Number of living cercariae		Tube No.	Number of living cercariae		Tube No.	Number of living cercariae	
8–13 ..	1	95	0	4	118	106	7	95	83
4– 8 ..	2	100	0	5	108	96	8	105	95
0– 4 ..	3	70	0	6	71	65	9	74	66

* Serum used in a final dilution of 1 : 6.

3. *The effect upon individual cercariae.* The cercariae are much altered in appearance as the result of contact with the serum. The movements of the body and tail soon become unnatural, the organism appearing to writhe spasmodically. With *Cercaria physae,* the angle in the fork of the tail early becomes less than ninety degrees. Later, the tips of the fork tend to curl and wither, so that locomotion is seriously impeded. The glands and other structures in that part of the body just behind the eye-spots generally retain their natural appearance longer than do other parts. Finally, the protoplasm takes on a vacuolated appearance and all signs of life are lost. By this time, the form can no longer be recognized as a cercaria save by observing with the high power of the microscope detailed elements which may persist. With some species of cercariae, for example, with the cercariae of *Diplostomum flexicaudum,* the tail is often dropped, yet the destructive action goes on in other species commonly without this effect.

The cercariae of the amphistome, *Diplodiscus temperatus,* were found to encyst readily on coming in contact with a cercaricidal serum. Generally, however, particularly when the serum was used in considerable concentration, the cyst wall was disintegrated and the encysted form was killed.[3] It should be mentioned that cat serum failed to cause the encystment of this cercaria.

SPECIFIC PROPERTIES OF THE SUBSTANCE IN THE SERUM RESPONSIBLE FOR THE CERCARICIDAL ACTION

We have been able to determine certain properties of the substance in serum responsible for the cercaricidal effect. It is apparently a highly labile substance, easily destroyed by heat or by desiccation, and quickly lost by the serum on storage. It can be absorbed from the serum, either with a mass of cercariae or with a mass of typhoid bacilli. Agencies which destroy any of the three known components of alexin also destroy the cercaricidal substance, although the cercaricidal substance is not to

[3] We are indebted to Prof. E. C. Herber, of Dickinson College, for observing and pointing out to us this phenomenon in our tests.

be confused in identity with the fractions of alexin. The experiments by which these points have been established will be described in detail.

1. *The effect of heat on the cercaricidal action of a serum.* To test the effect of heat on the cercaricidal action of serum, 0.5 cubic centimeter of the fresh serum of a herring gull was placed in each of eight tubes. These tubes along with tubes of the indicated control solutions were then corked and heated in a water bath at the temperatures and for the lengths of time given in Table 6. They were let cool to room tem-

TABLE 6.—*The effect of heat on the cercaricidal activity of serum**

Serum	Tube No.	Number of living cercariae	
		At start	(*Cercaria physae*) After 1 hour at 37° C.
Normal herring gull	1	45	0
Same, heated 1 hr. at 37° C.	2	33	0
Same, heated 10 minutes, at 47° C.	3	32	0
Same, heated 30 minutes, at 47° C.	4	23	0
Same, heated 60 minutes, at 47° C.	5	32	0
Same, heated 5 minutes, at 56° C.	6	29	15
Same, heated 15 minutes, at 56° C.	7	33	25
Same, heated 30 minutes, at 56° C.	8	31	26
Control solutions :			
0.75% NaCl, unheated	9	30	24
Tap water, unheated	10	31	33
0.75% NaCl, heated at 56° C. for 30 minutes	11	32	35
Tap water, heated at 56° C. for 30 minutes	12	40	38

* Tests made with 0.5 cc. of undiluted serum or 0.5 cc. of control solution plus 0.5 cc. of the cercaria suspension.

perature and 0.5 cubic centimeter of a suspension of *Cercaria physae* was added. The mixtures were incubated for one hour at 37° C. and the number of surviving organisms determined in the usual manner. The results are shown in Table 6. It can be seen from the data in Table 6 that the cercaricidal substance withstands heating at 47° C. for at least one hour, but is in considerable part destroyed by exposure at 56° C. for five minutes. Repeated tests corroborate the conclusion that heating a serum at 56° C. for 30 minutes destroys entirely the cercaricidal activity.

2. *The effect of storage on the cercaricidal action of serum.* Four cubic centimeters of fresh herring gull serum was placed in an electric refrigerator compartment at 5° C., immediately after the blood had clotted and the serum was pipetted off. The serum was titrated against *Cercaria physae* at this time, and after 24 and 48 hours. It was found that the cercaricidal action was lost entirely after 48 hours, the test being carried out with the undiluted serum and the cercaria suspension mixed in equal parts. A sample of fresh human serum, kept at room temperature, also was tested daily for cercaricidal action upon *Cercaria bessiae*. The serum had lost entirely its cercaricidal power by the fourth day.

3. *The effect of desiccation on the cercaricidal action of serum.* One-half cubic centimeter of herring gull serum was placed in a Syracuse watch glass in a calcium chloride desiccator at room temperature. After 24 hours, the dried serum was suspended in 0.5 cubic centimeter of distilled water and the suspension tested for cercaricidal action. It was found that only very little of the original cercaricidal activity remained. It is thus shown that the cercaricidal substance is in large part inactivated by desiccation.

4. *The absorption of the cercaricidal property of a serum.* We have attempted to absorb from a fresh serum the substance responsible for the cercaricidal action. For this purpose we have employed a dried mass of *Cercaria physae* as well as a dried mass of bacteria, *Eberthella typhosa.* The mass of cercariae was obtained by centrifuging down the 24-hour shed of 16 snails (*Physella gyrina*) giving off *Cercaria physae,* the final centrifugate being dried for 24 hours at room temperature in a calcium chloride desiccator. In the absorption test, four-tenths cubic centimeter of fresh duck serum was added to the dried cercariae and the mixture corked and kept at room temperature for three hours. The growth from thirteen 48-hour agar slants of a smooth strain of *Eberthella typhosa,*[4] centrifuged down and dried in a calcium chloride desiccator for 24 hours at room temperature, provided the mass of bacteria used for absorption. Five-tenths cubic centimeter of the same fresh duck serum was added to the bacterial mass and left in contact at room temperature for 3 hours. The normal serum and, after centrifugation, the two samples of serum absorbed with cercariae and with bacteria, respectively, were titrated for cercaricidal action against *Cercaria physae.* The results of the absorption tests show that it is possible to remove the cercaricidal substance from a fresh serum either with a mass of cercariae or with a mass of bacteria. The data are shown in Table 7.

5. *The relationship of alexin with the cercaricidal action of serum.* We have mentioned already the fact that when a serum is heated at 56° C. for 30 minutes, the cercaricidal power is lost. Because the alexin of the serum also is inactivated by exposure at this temperature for this period, we have investigated further the relationship between the cercaricidal action of serum and alexin. We have first inactivated separately each of the three known components of alexin and determined in each case the effect upon the cercaricidal action.

Five-tenths cubic centimeter of fresh duck serum was put in each of four tubes. One of these was kept as the normal serum control, the second was heated at 56° C. for 30 minutes to inactivate the thermolabile

[4] Obtained through the kindness of Mr. P. S. Strong, of the Department of Bacteriology, The College of Physicians and Surgeons.

TABLE 7.—*The absorption of the cercaricidal substance from the serum*

Serum dilution (final)	Cercaricidal Tests: Fresh Duck Serum — Tube No.	Number of living cercariae: At start	After 1 hr. at 37° C.	Duck serum absorbed with *Cercaria physae* — Tube No.	Number of living cercariae: At start	After 1 hr. at 37° C.	Duck serum absorbed with *Eberthella typhosa* — Tube No.	Number of living cercariae: At start	After 1 hr. at 37° C.
1– 4 ...	1	13	0	7	13	12	13	20	13
1– 8 ...	2	10	0	8	13	15	14	18	17
1– 16 ...	3	15	1	9	13	14	15	15	13
1– 32 ...	4	15	13	10	10	11	16	13	15
1– 64 ...	5	13	13	11	13	12	17	12	12
1–128 ...	6	11	13	12	18	15	18	10	11
Control solutions 0.75% NaCl	19	10	9						
Tap water ...	20	11	11						

Alexin Tests: Serum or control solution	Tube No.	Results 1 hr. at 37° C.
Fresh Duck serum	1	C
Duck serum absorbed with *Cercaria physae* ..	2	O
Duck serum absorbed with *Eberthella typhosa*	3	O
Control : 0.85% NaCl	4	O

Alexin tests : 0.1 cc. serum + 0.1 cc. senstized sheep cells + 0.3 cc. 0.85% NaCl.
C : complete ; O : negative.

element of alexin, the third was treated with yeast cells for 3 hours at 37° C. to inactivate the "third" component of alexin, and the fourth was treated with ammonium hydroxide for 3 hours at 37° C. to inactivate the "fourth" component. After the yeast cells were centrifugated down from tube three and the mixture in tube four had been neutralized by adding hydrochloric acid, all of the serums were treated for cercaricidal and alexic activity.[5] The results are shown in Table 7. It is indicated that the procedures which inactivate any of the three components of alexin also inactivate the cercaricidal power of a serum.

It remained to find whether or not the inactivation of the cercaricidal power resulted from destruction of the cercaricidal substance itself or was consequent upon the inactivation of alexin alone, the presence of

TABLE 8.—*The relationship between the cercaricidal action of serum and alexin*

Serum or control solutions	Cercaricidal tests*			Alexin tests†	
	Tube No.	Number of living cercariae		Tube No.	Result after 1 hr. at 37° C.
		At start	After 1 hr. at 37° C.		
Normal rat serum	1	30	0	11	C
Heated rat serum (56°C. ½ hr.)	2	35	33	12	O
Yeast-treated rat serum	3	43	40	13	O
Ammonia-treated rat serum ...	4	40	40	14	O
Normal cat serum	5	35	38	15	C
Heated rat serum + normal cat serum	6	45	43	16	C
Yeast-treated rat serum + normal cat serum	7	36	35	17	C
Ammonia-treated rat serum + normal cat serum	8	30	28	18	C
Control solutions: 0.85% NaCl	9	35	33	19	O
Tap water	10	25	20		

* In cercaricidal tests, serums employed in dilution of 1 : 6. When two serums are used, they are mixed in equal parts, the final dilution of the mixture being 1 : 6.

† In alexin tests : 0.1 cc. serum + 0.1 cc. sensitized cells, + 0.3 cc. 0.85% NaCl. When two serums are mixed, 0.05 cc. of each is employed. C : complete hemolysis ; O : no hemolysis.

alexin being essential for the function of the cercaricidal substance. We, therefore, added fresh cat serum which is known to possess alexin but to lack cercaricidal action, to the inactivated serums. Although the mixtures were found then to exhibit alexic activity, they failed still to manifest the cercaricidal effect. It is concluded from this that the cercaricidal substance is inactivated independently by the same agencies which destroy alexin. It has not yet been determined, however, whether

[5] The sheep cells and sensitizer employed in this work were supplied through the kindness of Dr. R. L. Kahn, of the University Hospital, Ann Arbor, Michigan.

the presence of alexin is required for the cercaricidal action. It should be pointed out that the alexin is fixed during the absorption of the cercaricidal substance from a fresh serum by cercariae or by bacteria (see Table 7.

DISCUSSION

The question of whether or not the cercaricidal action of the serum is related to the susceptibility or resistance of a given vertebrate to infection with a specific cercaria is of both theoretical and practical importance. Should the action of the serum occur as well in the blood plasma of the living vertebrate body, it seems quite probable that forms of cercariae which are killed in the test tube by serum from a given species of vertebrate would be unable to infect that species. If this action is the same both in serum and in plasma, and if a correlation does exist between the activity of the serum upon and the resistance of a given vertebrate to infection with a species of cercariae, the cercaricidal test with serum would assume importance in the selection of vertebrate hosts in experiments to complete trematode life history studies. When the fact is considered that the cercaricidal test can easily be carried out in a few hours, whereas the test for animal infection requires weeks or months, the advantages in time at least of the former, should it prove useful for the purpose, become apparent. Although we have not as yet studied this important question specifically, certain points in the data presented in Tables 1 and 2 throw some light on the promise which the cercaricidal test of the serum holds for this purpose.

In the first table, it is shown that against the strigeid *Cercaria physae*, of which unfortunately the life cycle is not yet established, the cercaricidal titers of human, rat, and duck serums are, respectively, 1 : 48, 1 : 198, and 1 : 24. This parasite probably, like other strigeids, invades a bird form and not a mammal, although, from this test with duck serum, one might infer that the duck could not serve as a host, since its serum is about as effective against the cercaria as that of man. Nevertheless, such difference as exists in these cercaricidal titers indicates a greater resistance of the cercaria to the blood of birds, some species of which, probably, it parasitizes, than to that of mammals, which it does not affect.

A marked difference in effectiveness is shown when these same three serums are titrated against the cercaria of *Schistosomatium douthitti*. None of the three serums is able to kill cercariae of this species. The parasite is known to invade and develop readily in the rat, and to penetrate into the skin of man, causing in the latter the well-known schistosome dermatitis or swimmer's itch, although it is probably unable to develop further in man. The parasite is, however, not infective for

ducks (Price), despite the failure of the serum of ducks to kill this cercaria. In this last reaction, the cercaricidal test appears definitely to fail as a means of selecting potential hosts of cercariae.

Tests were carried out with the same three serums against another newly described schistosome cercaria, whose life cycle has not yet been determined, *Cercaria stagnicolae* Talbot. Against this form, rat serum showed a titer of 1:48, human serum 1:24, and duck serum had no effect. It will be interesting to observe whether the definitive host of this parasite is determined eventually to be a bird or a mammal.

Several points brought out in Table 2 also deserve mention here. The serum of the green frog (*Rana clamitans*) is active against the cercaria of *Diplodiscus temperatus,* a parasite of the green frog, and, contrariwise, the serum of those mammals tested are not effective upon the cercariae of *Diplostomum flexicaudum* and *Cotylurus communis,* strigeids which do not parasitize mammals. Finally, it is improbable that the cat is susceptible to infection with the many forms which its serum fails to destroy.

The facts which have been presented indicate that any correlation which there may be between the resistance of an animal to infection with cercariae and possession of the cercaricidal substance in the serum will be only of a broad and general character and not of a narrow and limited specificity. If, however, even such a general relationship can be proved to exist, considerable assistance may be realized in the determination of potential hosts of cercariae in the trematode life histories.[6]

We should like to call attention to one consideration that may prove significant for workers who may in the future attempt studies upon the relationship of the susceptibility of a given vertebrate to infection by cercariae and the cercaricidal action of the serum. It seems probable that such relationships could be determined best by studies upon those cercariae which invade directly the blood stream of their vertebrate hosts —namely, the schistosome cercariae. Under this circumstance, the full effect of the blood is directed against the precise stage of the parasite which is infective for the vertebrate host. With many forms, for example, the intestinal trematode parasites, a metacercarial stage intervenes during which significant changes in the parasite may develop. It is hoped, however, that studies in this important relationship of the cer-

[6] In some respects these findings are similar to the results of Gengou (1899) who, from a study of the immunity to the bacterial infection anthrax, concluded that no correlationship existed between the refractory state toward anthrax and the presence of anthracidal substances in the serum. The immunity which man enjoys against the trypanosomes pathogenic for animals also seems to depend on factors other than the trypanocidal activity of the human serum (Culbertson, 1934).

caricidal substance of the serum to the resistance of hosts will be undertaken eventually with all of the groups of cercariae.

It is perhaps well to point out that the cercaricidal action seems distinct from the opsonizing and agglutinating antibodies of the normal serum since it goes on in the complete absence of the formed elements of the blood and without clumping or agglutinating the parasites which it affects. It does appear, however, to bear some relationship to the bactericidal power of the serum, since both the cercaricidal and bactericidal properties are manifested by the normal serum of many species of animals, are highly labile, and are removed from a serum by contact with bacterial cells.

It seems possible, finally, that the cercaricidal action of serum which we have described will help to explain the nature and origin of normal antibodies in general. Two schools of thought have developed to explain the appearance of normal bactericidal or virucidal substances in the serum, some workers believing that the substances responsible for these activities arise in response to exposure to or contact with the specific bacteria or virus upon which the serum acts, while other investigators feel that the action is relatively non-specific and that the property of the blood develops more or less spontaneously during the normal maturation of the individual (Jungeblut and Engle, 1932). The author (Culbertson, 1935), in a recent review of the literature upon one of the antagonistic properties of the normal serum of man—namely, the trypanocidal activity, pointed out that sub-clinical infection or specific exposure through other channels probably cannot account for the presence of the trypanocidal substance in the blood of all normal human beings. It seems equally true that the occurrence of the cercaricidal substance in the serum of man and other animals likewise cannot be explained on the basis of specific immunization, for the substance is found in animals which are at no time in life exposed to either the specific parasites against which their serum is found to be effective or other forms which are closely related.

CONCLUSIONS

1. An antagonistic action of the normal serum of vertebrates against larval trematodes (cercariae) has been described. The action is manifested by the serum of representatives of all classes of vertebrates. A considerable number of species of cercariae belonging to different groups have been tested and found susceptible to the cercaricidal action of the normal serums.

2. The substance in the serum responsible for the cercaricidal effect is highly labile, being readily destroyed by heat or by desiccation and quickly lost in storage. It can be removed from the serum by absorption

with a dry mass of either cercariae or bacteria. The alexin of the fresh serum is fixed during the process of absorption of the cercaricidal power by these substances. Agencies which destroy specifically the several known components of alexin also destroy at the same time the cercaricidal power of the serum.

3. It is possible that with further work the test for cercaricidal action of serum may aid in determining potential hosts of cercariae in trematode life histories. The relationship of the cercaricidal action to some other antagonistic properties of serum, and the limited possibility of its development as a response to specific immunization are briefly discussed.

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THE DEVELOPMENT OF NATURAL AND ARTIFICIAL RESISTANCE OF YOUNG RATS TO THE PATHOGENIC EFFECTS OF THE PARASITE *TRYPANOSOMA LEWISI** †

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The common parasite of the rat, *Trypanosoma lewisi,* although considered by many to be non-pathogenic, has on various occasions been found to be pathogenic. Brown (1914) found that the virulence of a strain of *T. lewisi* might so change that at one time it would produce rapidly fatal infections and later lose its virulence to regain it again at a later time. Roudsky (1911) also observed that the virulence of *T. lewisi* was variable and that, by giving massive infections and by rapid passage he could increase the virulence to a point where it was pathogenic even to mice. Jurgens (1902) noted that at times a strain of *T. lewisi* might prove to be pathogenic to rats and observed further that the infection was more commonly pathogenic to young than to old rats.

A wild strain of *T. lewisi* brought into our laboratory was found to be pathogenic to young and not to old rats. Furthermore it was found that its virulence did not fluctuate to any appreciable extent; it never became pathogenic to old rats and remained pathogenic to young ones. Since such a high percentage of the young animals were killed by this wild strain of trypanosomes it was thought to be particularly virulent. Another strain of *T. lewisi* was therefore secured that had been maintained in laboratory animals for several years. This strain, however, was just as pathogenic to young animals as was the wild strain. This finding presented the problem of determining the age at which the young rats became resistant to the pathogenic effects of this parasite.

The parasite used in these studies was a strain that had been maintained for several years in laboratory rats. The animals used were ordinary white rats that were in good condition when started on the experiment; they were given a ration suitable for rapid growth and development and were given excellent care throughout the experiment. The test animals were separated into groups of various ages ranging

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from two to forty days. In every case except in the two-day-old group the infected and control animals were all male litter mates. The inoculations were made intraperitoneally and the number of trypanosomes given varied according to the weight of the animal.

The rats were divided into two categories (1) those that were allowed to suckle their mothers and (2) those that were weaned. The suckling young were divided into four groups. (See Table 1.)

TABLE 1.—*Showing the death rate in a group of rats infected with* T. lewisi *and their uninfected litter-mate controls.*

	Suckling				Weaned		
	Experimental						
Age in days	2	10	20	30	20	30	40
No. of rats	10	20	50	25	118	25	25
No. of deaths	0	0	2	0	100	19	0
% which died	0	0	4	0	84.7	76	0
	Uninfected controls						
Age in days	2	10	20	30	20	30	40
No. of rats	0	20	43	0	98	25	0
No. of deaths	0	0	0	0	3	1	0
% which died	0	0	0	0	3.1	4	0

All of the parasitized animals ran a normal infection as described by Taliaferro (1929). At the peak of the infection large numbers of trypanosomes were present in the peripheral blood but none were seriously affected by the parasites. From the 105 infected suckling rats only two died and one of those died before the infection reached the peak. The death rate in the infected group that was weaned was high being 84.7 and 76 per cent, respectively for the twenty and thirty-day-old groups. The death rate among the litter mate controls for these groups was 3.1 and 4 per cent, respectively. The parasites were obviously pathogenic for rats not over thirty days old. By the second week the majority of the infected animals could be selected from the controls by their appearance alone. They were smaller and were rougher in appearance, and by the beginning of the third week they were obviously weaker than the controls. Most of the deaths occurred during the third week following inoculation, after the number of trypanosomes in the blood had reached a low level as compared to the number present at the peak of the infection.

The virulence of the strain of *T. lewisi* used fluctuated but slightly during the year it was being studied. A total of 118 rats were infected when twenty days old and the death rates in the various groups ranged from 80 to 92 per cent (see Table 2).

The animals infected when 40 days old survived in all cases and suffered little, if any, from the infection.

From these data it seems that there is a very definite age resistance developed by the young rats to the pathogenic effects of our strain of

TABLE 2.—*Showing the death rate of weaned rats infected with* T. lewisi *when 20 days old.*

Date	1/7/31	3/30/31	5/19/31	10/12/31	11/6/31	2/14/32
No. of animals	25	25	20	22	11	15
No. of deaths	23	20	16	19	10	12
% which died	92	80	80	86.3	90.9	80

T. lewisi and that the resistance is well established by the time the rats are forty days old. It is also apparent that for some reason, which cannot be explained at this time, the suckling rats were protected from the effects of the parasites. They were not made immune to infection, for all became infected and showed a large number of trypanosomes in their peripheral blood. Furthermore the mothers were shown not to be immune, as they were successfully infected at the close of their lactation period. That *Bartonella* sp. was not responsible for the death of the young rats seems evident for the litters were not separated until infected and repeated attempts were made to diagnose the presence of *Bartonella* even in splenectomized animals, without success.

Owing to the fact that the development of age immunity was so definite, an attempt was made to artificially increase the resistance of the young rats so they would not be affected by trypanosomes. This was successfully done in two ways, (1) by implanting the pituitary glands of adult non-immune rats intramuscularly into the young susceptible animals, and (2) by injecting oestrin subcutaneously into the young rats.

The young rats receiving the pituitary implants were twenty days old when infected and received their first implant. The pituitary gland was removed from a non-immune adult and immediately implanted by means of a small trocar intramuscularly on the median surface of the hind leg of a young rat. These implants were made in ten infected twenty-year-old rats and none of these rats died and they showed no symptoms of the infection. The nine brother controls for the animals receiving the pituitary implants, however, suffered from the infection and 8, or 88.8 per cent, died during the first three weeks of the infection.

The animals receiving oestrin subcutaneously were able to withstand the infection of *T. lewisi* much better than did their brother controls. In this experiment twenty rats, twenty days old were given two rat units of oestrin daily for two weeks following their infection. Of this number only one animal, or 5 per cent, died while from a group of 17 brother controls for these rats fourteen, or 82.3 per cent, died during the first three weeks of the infection. Just why the implanting of pituitaries or the injection of oestrin into young rats made them more resistant to the effects of an infection of *T. lewisi* cannot be answered at this time. Work along this line and work to determine if it will be possible to

increase the resistance of other animals to their parasites by this and similar means is being carried on at this time.

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A REVIEW OF THE STATUS OF THE REPTILIAN NEMATODES OF THE GENUS *RHABDIAS* WITH A REDESCRIPTION OF *RHABDIAS FUSCOVENOSA* VAR. *CATANENSIS* (RIZZO, 1902) NEW RANK*

Tso-chih Chu

In the course of studies on the biology of *Rhabdias* the writer experienced some difficulty in making a specific identification of the principal form used. These worms obtained from the lungs of snakes were first identified as *Rhabdias fuscovenosa* (Railliet, 1899) Stiles and Hassall 1905, but upon careful study of Railliet's description and Goodey's (1924) redescription certain discrepancies were found and in most characteristics the writer's *Rhabdias* seemed to be closer to the form described as *Strongylus catanensis* by Rizzo (1902) which Chitwood (1933) synonymized with *R. fuscovenosa.* These two, however, are almost indistinguishable from a number of other described reptilian *Rhabdias.* Accordingly the author has reviewed the descriptions of the reptilian forms similar to the first described species, *R. fuscovenosa* (Railliet, 1899) Stiles and Hassall 1905, in order to place satisfactorily the form under consideration. Those from amphibians are not considered here since the writer's form could not be experimentally established in amphibian hosts and there is no evidence of cross infection with *Rhabdias* from reptile to amphibian hosts or vice versa.

The confusion in classification of *Rhabdias* seems to be due (1) to the absence of a parasitic male, (2) dearth of distinct morphological differences in the parasitic "female," (3) inadequate descriptions of certain forms, (4) incomplete information on the life history with the resulting lack of knowledge on the morphology of the free living generation. Consequently if worms recovered from new hosts or in new localities vary from those described they are likely to be described as distinct species. Most commonly used characteristics in this speciation are the size of the worms, the position of the reproductive organs, the

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relative number of eggs in the uteri, the shape and size of the tail, the oral opening and the buccal capsule. The fact that the host origin and the size of the worms are unsatisfactory bases for setting up species is shown by the writer's experience with the form he has identified as *R. fuscovenosa* var. *catanensis.*

These worms were recovered, as natural infections, from the lungs of a large percentage of garter snakes, *Thamnophis sirtalis,* examined. They ranged in size from 2.43 to 4.99 mm. Morphologically identical forms within the limits of the above measurements were less commonly found in *T. sauritus, T. ordinoides, Natrix septemvittata, Storeria dekayi, S. occiptomaculata, Lampropeltis triangulum* and *Liopeltis vernalis.* Forms morphologically identical but smaller (1.62 to 3.30 mm.) were nearly as common in *Natrix sipedon* as in *Thamnophis sirtalis.* The incidence of infection in the above listed snakes in three areas in U. S. A. is given in table 1. It is interesting to note here that with this apparent lack of host specificity yet these worms have not been reported from the few poisonous snakes examined. The writer examined three rattle snakes, *Sistrurus catenatus catenatus,* and one copperhead, *Agkistrodon mokasen,* with negative results. A strain recovered from *T. sirtalis* was carried in the laboratory and in the course of life history studies (Chu, 1936) successfully transmitted to previously uninfected usually laboratory raised *T. sirtalis, T. sauritus, N. sipedon, L. triangulum, S. dekayi,* and *Coluber constrictor.* A strain recovered from *N. sipedon* was similarly transmitted to *N. sipedon* and *T. sirtalis.* The worms from these experimental infections were not all measured but those that were fell within the range of measurements already given and those from *N. sipedon* seemed to be for the most part smaller than those from *T. sirtalis.*

The uncertainty of size alone is further emphasized by table 2 which gives the available measurements for the reptilian species of *Rhabdias.* In fact on the basis of the data contained in table 2 it seems to the writer that the forms described as *R. fuscovenosa, R. catanensis, R. ophidia, R. annulosa* and the writer's form are probably all the same species, such differences as are brought out probably being at the most only variety or subspecies differences. It seems to the writer, however, desirable to maintain the individuality of these several worms by giving them variety rank. They may be physiological varieties of the same species or it is possible even that they are distinct species, The final explanation must await a more detailed study of the morphology of the parasitic adults and complete knowledge of the life history including, particularly, careful morphological studies on the free-living males. In the meantime the following classification is suggested:

TABLE I.—*Incidence of infections and average worm burden of R. fuscovenosa catanensis in various species of snakes from three different localities in the United States*

Host	Michigan			West Virginia			Maryland		
	Number examined	Per cent infected	Average worm burden	Number examined	Per cent infected	Average worm burden	Number examined	Per cent infected	Average worm burden
Thamnophis sirtalis	35	51.4	7.9	21	100.0	29.3	4	100	8.2
T. sauritus	5	100.0	7.4	2	100.0	48.0			
T. ordinoides				1	100.0	27.0			
Natrix sipedon	16	12.5	6.5	38	94.7	12.5			
N. septemvittata				4	100.0	2.2			
Storeria dekayi				7	100.0	9.5			
S. occipto-maculata	1	100.0	2				10	0	0
Lampropeltis triangulum	1	100.0	4	3	66.6	13.0			
Liopeltis vernalis	3	66.6	4.5	3	100.0	8.0			

TABLE 2.—*Comparative data on nematodes of the genus Rhabdias from snakes. All measurements given are in mm.*

Present name	*R. fuscovenosa* (Railliet 1899) Stiles and Hassall 1905		*R. (Strongylus) catanensis* (Rizzo 1902) Chitwood 1933	*R. ophidia* Goodey 1924		*R. annulosa* Hsu 1933
Suggested name	*R. fuscovenosa fuscovenosa*	*R. fuscovenosa brevicauda*	*R. fuscovenosa catanensis*	*R. fuscovenosa ophidia*	*R. fuscovenosa ophidia*	*R. fuscovenosa annulosa*
Authority for data given	Railliet 1899 Goodey 1924	Hsu and Hoeppli 1931	Rizzo 1902	Goodey 1924	Hsu and Hoeppli 1931	Hsu 1933
Host	*Tropidonotus natrix*	"Green snake"	*Tropidonotus natrix*	*Coluber leopardinus*	*Natrix tigrina*	*Zaocys dhumnades*
Locality	France and England	Szechuan, China	Catania, Sicily	London zoo, England	Nanking, China	Nanking, China
Body length	3–6	5.27	3–4	6.0–6.5	4.65–4.98	5.65–6.30
Body width	0.15–0.19	0.27	0.18	0.2–0.25	0.18–0.186	0.25–0.31
Buccal capsule	Infundibiliform	Infundibiliform	.....	Funnel shaped	Funnel shaped	Cup shaped with chitinous ring
Distance of nerve ring from anterior end	Midway of oesophagus	0.145	.....	0.2	0.14–0.143	0.146–0.162
Distance of excretory pore from anterior end	.....	0.225	.....	.....	0.222–0.253	0.212–0.260
Oesophagus length	0.27	0.336	0.23	0.3–0.33	0.312–0.328	0.310–0.320
Oesophagus width anterior	0.07	.....	.....	.....	.....	0.05–0.053
Oesophagus width middle	.....	.....	.....	.....	.....	0.044–0.049
Oesophagus width posterior	.....	.....	.....	.....	.....	0.062–0.068
Distance of vulva from anterior end	Anterior half of body	2.66	.....	3.0–3.2	2.05–2.23	2.29–2.56
Tail length	0.11	0.092	0.23	0.27–0.30	0.334–0.35	0.153–0.179
Egg length	0.079–0.085	0.06–0.068	0.100	0.098–0.1	0.07–0.073	0.063–0.068
Egg width	0.045–0.048	0.033–0.04	0.060	0.04–0.041	0.037–0.048	0.03–0.037
Number eggs per female	Few (?)	Few	Few	Few	Few	Few

TABLE 2.—*Comparative data on nematodes of the genus Rhabdius from snakes. All measurements given are in mm. (Continued)*

Present name	*R. labiata* Pereira 1927	*R. vellardi* Pereira 1928	*R. serpenticola* v. Linstow 1904	*R. (Sclerostoma) eustreptos* (MacCallum 1921) Chitwood 1934	*R. chameleonis* Skrjabin 1916	Present form
Suggested name	?	?	?	*R. eustreptos*	*R. chameleonis*	*R. fuscovenosa catanensis* Chu
Authority for data given	Pereira 1927	Pereira 1928	v. Linstow 1904	MacCallum 1921	Skrjabin 1916	*T. sirtalis*
Host	*Rhabinea merremi*	*Philodryas schotti; Oxhurropus trigeminus*	*Heterodon platyrhinus*	*Ophibolus getulus*	*Chameleon* sp.	*N. sipedon*, etc. (see text)
Locality	Brazil	Brazil	Germany	New York zoo, U. S. A.	East Africa	U. S. A.
Body length	2.2–2.4	3.0–3.3	4.49	7.0–8.0	24.0	1.62–4.99
Body width	0.11–0.13	0.12–0.20	0.24	1.0	1.0	0.082–0.167
Buccal capsule	None	None	.....	Present	0.02–0.034	Infundibiliform, small
Distance of nerve ring from anterior end	0.10–0.13	0.15	.....	.....	.....	0.120–0.147
Distance of excretory pore from anterior end	.....	0.035–0.040	.....	.....	.....	0.192
Oesophagus length	0.25–0.26	0.25–0.27	0.29	0.40	1.225	0.249–0.299
Oesophagus width anterior	0.046–0.048	0.053	.....	0.10	0.10	.....
Oesophagus width middle	.....	.....	.....	.....	.....	.....
Oesophagus width posterior	.....	.....	.....	.....	0.187	.....
Distance of vulva from anterior end	Middle	Middle	Anterior to middle	Middle	Posterior to middle	1.250–2.332
Tail length	0.15–0.19	0.13–0.14	.....	.....	0.34	0.14–0.23
Egg length	0.084–0.092	0.069	0.065	.....	0.136	0.068–0.084
Egg width	0.053	0.038–0.046	0.049	.....	0.076	0.040–0.049
Number eggs per female	Many	Many		Many		Few

Rhabdias fuscovenosa var. *fuscovenosa* (Railliet, 1899) new rank.
Including Railliets, (1899) and Goodey's (1924) forms.
R. fuscovenosa var. *catanensis* (Rizzo, 1902) new rank.
Including Rizzo's *Strongylus catanensis* (1902) and the writer's present form.
R. fuscovenosa var. *brevicauda* new variety.
Including Hsu and Hoeppli's *R. fuscovenosa* 1931.
R. fuscovenosa var. *ophidia* (Goodey, 1924) new rank.
Including Goodey's (1924) and Hsu and Hoeppli's (1931) *R. ophidia.*
R. fuscovenosa var. *annulosa* (Hsu, 1933) new rank.
R. eustreptos (MacCallum, 1921) Chitwood, 1934.
R. chameleonis Skrjabin 1916.

Of uncertain identity.
R. labiata (Pereira, 1927).
R. vellardi (Pereira, 1928).
R. serpenticola (v. Linstrow, 1904).

The species *R. fuscovenosa* including its five varieties would be characterized by the relative few eggs in the uteri and as far as our present information goes range in size from 1.62 to 6.5 mm. Railliet's form having been described first would utilize the species name for its variety name (*R. fuscovenosa* var. *fuscovenosa*). Goodey's (1924) form is apparently the same and his redescription is the basis for comparison with the others. Specimens resembling this were obtained from the same host in Italy by Rizzo and described as *Strongylus catanensis.* Although Rizzo's form is about the same size as *R. fuscovenosa fuscovenosa* it has a tail about twice as long. The eggs of Rizzo's specimens are also larger than those of the latter. It is hence considered here as a separate variety. As already noted the writer's variety has been identified as the same as Rizzo's A redescription of this variety on the basis of the author's material will be given later. The worm reported by Hsu and Hoeppli (1931) as *R. fuscovenosa* is from a different host, the "green snake" in China. Although the tail is reported to be only a little shorter than that in *R. fuscovenosa catanensis* the drawing emphasizes the shortness and gives it a different shape. The author coins the variety term *brevicauda* for it. The outstanding characteristics of *R. ophidia* as described by Goodey (1924 a) are the funnel-shaped mouth and the external irregular walls of the seminal receptacle. Hsu and Hoeppli (1931) do not accept this latter point. The tail as indicated in both these reports is strikingly long but of course may be related to the apparently larger size of the worms. For the present the writer would retain a variety distinction for these. *R. annulosa* (Hsu, 1933) falls within

the size range of those already discussed but the chitnous ring-like structure described in the anterior part of the buccal cavity is apparently unique in this group. That this alone is of specific importance the writer cannot accept so that he considers it as a variety of *R. fuscovenosa.*

Of the remaining forms two apparently are entitled on the basis of present information to specific identity. *R. austreptos,* described by MacCallum (1921) from *Ophibolus getulus* (= *Lampropeltis getulus*) as *Sclerostome eustreptos,* is distinguished from all the varieties of *R. fuscovenosa* by the large number of eggs in the uteri. *R. chameleonis* Skrjabin, 1916, described from *Chameleon* sp., is nearly four times as long as the maximum length recorded for any other species of Rhabdias described and should perhaps retain its specific rank on that basis.

Three other described species are so inadequately described as to be of doubtful standing. *R. labiata* Preiera, 1927, is described from *Rhabinea merremi* in South America. No specific differentiation is mentioned but measurements given would suggest that it is probably a variety of *R. fuscovenosa. R. vellardi* Peiera, 1928, from *Philodryas schotti* and *Oxhyrropus trigeminus,* is apparently a form with many eggs and hence distinct from *R. fuscovenosa.* On the other hand Harwood (1932) reports *R. vellardi* from *Heterodon contortrix, Storeria dekayi, Potamophis striatulus,* and *Thamnophis proximus* and in no case does he find more than a dozen eggs in a worm—since in these cases the uteri were not distended and others were entirely free of eggs Harwood leaves the impression that normally they should have contained many eggs. Further information it seems to me is needed before either of these South American forms or Harwood's form can be even provisionally identified. *R. serpenticola,* described by von Linstrow (1904) from *Heterodon platyrhinus* without figures, is apparently, judging by size, a variety of *R. fuscovenosa.* However, von Linstrow says "Die Art ist vivi par und die Embryonen erfallen zahlreich den Uterus." The status of this form then remains uncertain.

REDESCRIPTION OF *Rhabdias fuscovenosa* VAR. *catanensis* (RIZZO, 1902)

The redescription of this worm is based primarily on parasitic adults recovered from *Thamnophis sirtalis* and the free-living adults obtained by culturing from snakes known to harbor only this form. The writer feels the inclusion of the morphological characteristics of the free-living generation is essential to an ultimate classification of the species of the genus *Rhabdias.*

Life history.—The egg from the parasitic generation hatches into a rhabditiform larva which may develop directly into an infective larva or

it may develop into a functional living male or female. The eggs from the free-living generation produce rhabditiform larvae which develop into what appears to be infective larvae but all attempts at infection have failed so far. The direct type of development predominates (Chu, 1936).

Parasitic Adult:—2.43–4.99 mm. long (1.62–3.3 mm. from *Natrix sipedon*) by 0.84–0.167 mm. wide. Cuticula slightly but uniformly inflated. Oral opening small, subtriangular, and surrounded by six indistinct lips each bearing a small papilla on the inner circle. The papillae of the outer circle not observed. Amphids oval, slightly dorsal to lateral. Buccal cavity very small, funnel-shaped and without thick wall or teeth. Esophagus 0.249–0.299 mm. long having a minimum diameter at isthmus of 0.025–0.036 mm. and a maximum diameter at posterior enlargement of 0.044–0.068 mm. Nerve ring 0.120–0.148 mm. from anterior end of body. Excretory pore 0.145–0.155 mm. from the anterior end of body. Intestine without any marked enlargement or constrictions. Anus 0.140–0.233 mm. from posterior end of body. Tail attenuated, caudal process usually not extending through the cuticular inflation. Vulva approximately in the middle of the body. Anterior ovary extends to within 0.369–0.899 mm. of the anterior end of body and the posterior ovary extends to within 0.533–0.966 mm. of the posterior end of body. Eggs varying in number from a few to not more than a hundred, 68 to 84 long by 40 to 49 μ wide. (Figs. 1, 2, 3, and 4.)

Free-Living Male:—0.556–0.816 mm. long by 0.032–0.052 mm. wide. Buccal cavity 0.012 mm. in depth. Esophagus consisting of a corpus 0.080 by 0.016 mm. in the anterior part and 0.020 mm. at the pseudo bulb, and isthmus 0.040 by 0.010 mm. and a valvular bulb 0.022 by 0.020 mm. Anus 0.032–0.052 mm. from the posterior end of body. Tail truncated with 10 pairs of caudal papillae, 3 of which are pre-anal, 1 adanal, and 6 postanal. The sixth and eighth pair of papillae are sublateral while the remainder are ventral. Spicules 0.032–0.036 mm. long by 0.004–0.008 mm. wide, cephalated and reaching their maximum diameter approximately 77.5% of their length from the proximal end. Gubernaculum 0.016–0.036 mm. long. (Figs. 5 and 6).

Free-Living Female:—0.816–0.866 mm. long by 0.049–0.058 mm. wide. Buccal cavity 0.016 mm. in depth. Esophagus consisting of a corpus 0.056 mm. long with an anterior diameter 0.016 mm. and a posterior diameter 0.026 mm. and isthmus 0.048 mm. long by 0.020 mm. wide and a posterior vulvalate bulb 0.036 by 0.028 mm. Nerve ring 0.072 mm. from anterior end, excretory pore slightly posterior to nerve ring. Anus 0.048–0.056 mm. from posterior end of body. Tail attenuated. Vulva 0.483 mm. from anterior end of body; anterior ovary extending to within 0.220–0.250 mm. of anterior end of body and posterior ovary extending to within 0.188–0.195 mm. of posterior end of body. Eggs 56 to 59 by 24 to 30 μ wide; a few eggs in each horn of uteri, development in uterus lacking or partial, rarely complete. Eggs thus in one celled to tadpole stage, rarely containing a larva, when laid. (Fig. 7).

Hosts: Most frequently found in *Thamnophis sirtalis* and *Natrix sipedon.* Also occurred naturally in *Natrix (Tropidonotus) natrix,** *Natrix septemvittata, Storeria dekayi, S. occiptomaculata, Thamnophis sauritus, T. ordinoides, Lampropeltis triangulum* and *Liopeltis vernalis. Coluber constrictor* experimentally infected.

Location: Lung.

*First report, by Rizzo (1902).

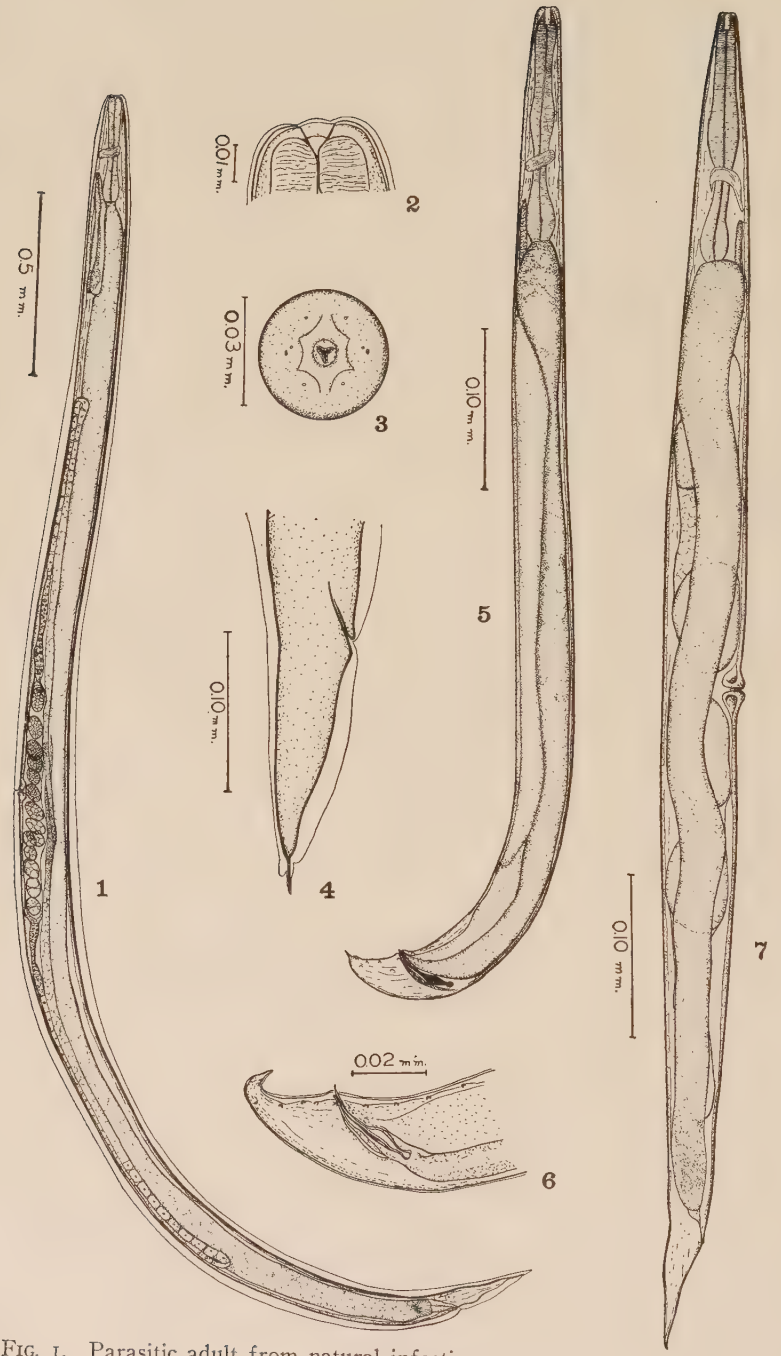

FIG. 1. Parasitic adult from natural infection.
FIG. 2. Cephalic end of parasitic adult showing character of buccal cavity.
FIG. 3. End face view of parasitic adult showing the arrangement of lips and papillae.
FIG. 4. Posterior end of parasitic adult.
FIG. 5. Free living adult male, 5 days after hatching.
FIG. 6. Posterior end of free living adult male.
FIG. 7. Free living adult female, 5 days after hatching.

Locality: Italy* and Maryland, Michigan, and West Virginia in the U. S. A.

Specimens: U. S. Nat. Mus. Helminth. Coll. Nos. 39557 and 39558.

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STUDIES ON THE LIFE HISTORY OF *RHABDIAS FUSCOVENOSA* VAR. *CATANENSIS* (RIZZO, 1902)*

Tso-chih Chu

INTRODUCTION

Since Metchnikov (1865) and Leuckart (1865)[1] first reported heterogony, *i.e.,* the occurrence of a free living sexual generation, in the life history of *Rhabdias bufonis* there has been considerable interest in the biological significance of the life history of this and related forms. Schaake (1931) reviewed the literature on and experimentally studied this form and concluded that only the heterogenous type of development occurred. Schneider (1866), Travassos (1926), Goodey (1924), and others have described the free living adults of other species of *Rhabdias* from amphibians and reptiles. Walton (1929) was the first to report direct development, *i.e.,* the development of infective larvae from the eggs of the parasitic generation without the interposition of a free living sexual generation, in an amphibian *Rhabdias.* In this case (*R. ranae*) only the direct type of development occurred in Walton's experience.

On the other hand with reptilian species of *Rhabdias* the direct type of development alone is reported by most authors (Railliet, 1899; Goodey, 1924, and others). However, Periera (1927 and 1928) in describing *R. labiata* and *R. vellardi* from South American snakes records only the heterogenous type of development. So far as the author is aware there is no published report of the finding of both types of development in any given species of *Rhabdias.* Chitwood (unpublished)[2] has found both types of development in *R. ranae, R. eustreptos,* and *R. fulleborni* and the writer has also seen the concurrent direct and indirect development in the two former species and in a reptilian form identified by the writer (Chu, 1936) as *R. fuscovenosa* var. *catanensis* (Rizzo, 1902). It is the purpose of this paper to present the results of experimental studies on the life history of this latter form.

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The writer is indebted to Drs. W. W. Cort and G. F. Otto under whose direction this work was carried out. He is also indebted to Dr. George R. La Rue for the use of laboratory facilities at the University of Michigan Biological Station.

Acknowledgement is also made to the China Foundation and the Rockefeller Foundation for fellowships (1931–33 and 1933–34 respectively) which made these studies financially possible.

[1] Concerning the controversy between Metchnikov and Leuckart see Quart. Jour. Micro. Sc., N. S. 6: 101, 1866.

[2] The writer is indebted to Dr. B. G. Chitwood for permission to quote from his unpublished manuscript on the genus *Rhabdias.*

MATERIALS AND METHODS

General. The worms used were obtained from snakes, *Thamophis sirtalis,* collected in three different localities Cheboygan County, Michigan; Randolph County, West Virginia; and Baltimore, Maryland; and *Natrix sipedon* collected from the two former regions.

All snakes for experimental purposes and host examinations were kept in wooden boxes 36 × 16 cms. in size. The tops of these cages and the small holes at the ends were covered with wire screen so that air could circulate freely. On the inner bottom surface of each cage was spread a thin layer of paraffin (or paint) to prevent the absorption of water by the wood. In a further attempt to keep these cages dry only a small quantity of water in a shallow dish was given to the snakes. Any water getting on the non-absorbent surface of the cage would evaporate rapidly and the feces become dry in a short time killing all eggs and larvae contained. The cages were cleaned frequently, *i.e.,* as often as debris accumulated. Under such conditions there was never any evidence of accidental infection of the experimental snakes.

In some cases snakes, parasite free when captured were used for experimental purposes. Such animals were isolated in the above described cages and were only considered as free of *Rhabdias* infection when repeated cultures of feces were negative over at least a three week period. This time permitted the maturing of any infections which might have been acquired shortly before the capture of the snakes. For most of the experiments, however, *Natrix sipedon* and *Thamnophis sirtalis* were reared parasite free in the laboratory. Towards this end gravid females were captured and isolated until the birth of the young. Upon birth the young snakes were removed and raised with only a few in each box. As an additional precaution such animals were examined, by culturing the feces, before using them in experimental work.

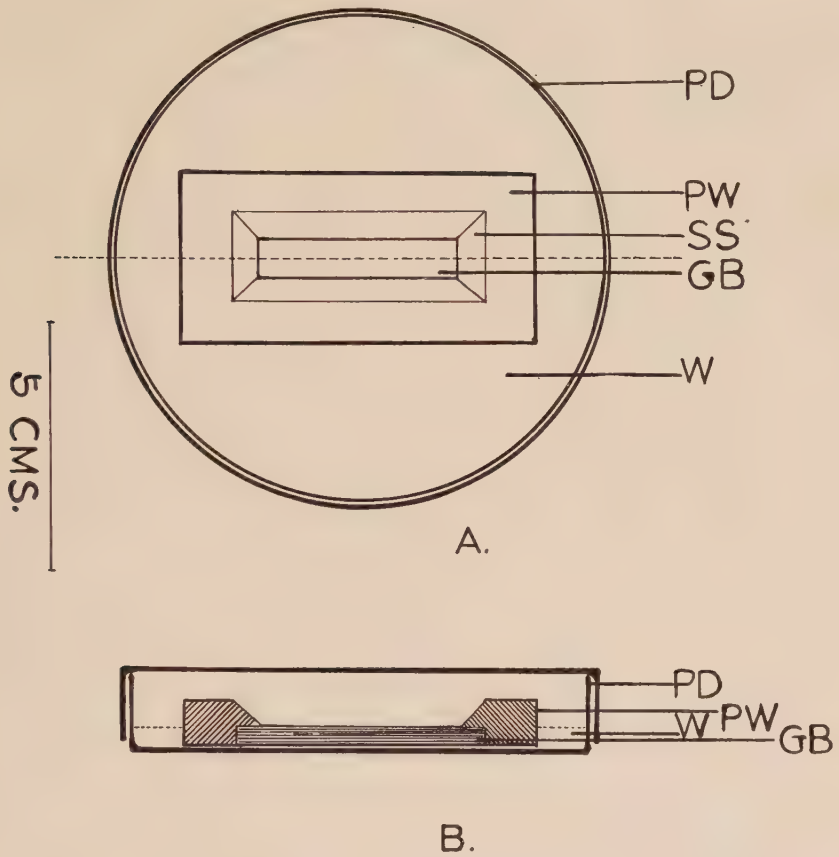
Natural food for experimental animals was avoided because of the possible danger of infection with *Rhabdias.* Fresh fish was used as food for *Natrix sipedon* and *Thamnophis sirtalis.* Snakes having more restricted food habits were rarely used experimentally and to these animals only food known, by direct examination, to be free from infective larvae of *Rhabdias* was fed. Forced feeding was often necessary to keep the snakes alive. Furthermore regularity in feeding was absolutely essential to insure a relatively constant fecal output since elimination of waste is very slow, requiring 4 or 5 days from the time of feeding. It was often necessary to extract fecal material for examination by pressing the abdomen near the anal opening.

Fecal examinations of experimental animals were made by dilution count (modification of Stoll's 1923 method) in heavy infestations, direct

count of eggs or larvae in lighter infections, and by culture methods. No fecal specimen was considered as negative unless it failed to produce larvae on culturing. The special culture methods used will be given later.

The parasitic stages of the worms were recovered by direct examination of the animals after killing them by asphyxiation with xylol fumes or by decapitation. All organs were removed and teased apart in physiological saline for examination except the rectum which was similarly handled in tapwater. In removing the lungs care was taken to include a portion of the trachea and all of the membranous lower lung except when worms and eggs were being procured for culture purposes when only the thick tubular part of the lung was removed.

Culture Methods and Technique. Since the methods and technique of culturing the free-living stages of *Rhabdias* have an important bearing on the results obtained it seems desirable to outline them in some detail. In the beginning of this work the writer made use of moist charcoal-feces cultures and various liquid cultures. Metchinokov (1865), Goodey (1924), Fulleborn (1928) and others have preferred the moist charcoal-feces or sand-feces mixtures over liquids for the culturing of both reptilian and amphibian *Rhabdias.* In the writer's experience, however, the results of such cultures proved to be too variable for quantitative work. The principal difficulty seemed to be in maintaining a uniform degree of moisture within the cultures and from culture to culture. An attempt was, therefore, made to standardize liquid cultures. The best results were obtained when only a film of liquid was present. This was difficult if not impossible to maintain constantly in glass containers. About this time it was observed that when moist feces were spread on the surface of unglazed porcelain some larvae always developed. Experiments showed that the surface of plaster of Paris casts were equally suitable and further that chambers with plaster of Paris sides and glass bottoms would serve. Such containers permitted the use of transmitted light in microscopical examination and with the aid of a metal mold could be made easily and quickly. The chambers (text-fig. 1) having inner measurements of 4 x 1 x 0.75 cm. and outer measurements of 7 x 3.25 x 1 cm. could be placed in a 10-cm. petri dish. When 25 cc. of water was added to the petri dish which was then covered, all surfaces of the chamber were kept moist and had a pH of about 7.6. Such chambers could be removed from the petri dish, for examination, and returned without materially affecting the cultural conditions. Counts could be made of eggs or worms directly without the error involved in dilution counting or other sampling methods. Eggs or larvae inoculated into such chambers with feces to furnish bacteria for food or with pure cultures of bacteria generally yield better than 50 per cent development.



TEXT-FIG. 1. A. Diagram of the plaster culture chamber. Top view. B. Cross section of same. Note the relative heights of the plaster chamber, dish, and the piece of glass inserted in the bottom of the chamber. PD = petri dish; PW = plaster wall; SS = slanting side; GB = glass bottom; W = water.

Such culture chambers were also useful, as will be shown later, in isolating infective larvae from other free-living stages.

The above culture chambers while useful were not satisfactory for studying the suitability of various bacteria as food. For this purpose nutrient agar (5 cc. ordinary nutrient agar diluted with 1 cc. water) in the bottom of cotton stoppered 75 cc. Erlenmeyer flasks was used. The technique of handling will be given under the experiments concerned.

OBSERVATIONS AND EXPERIMENTAL RESULTS

The Parasitic Adult. Examination of snakes naturally infected with *R. fuscovenosa catanensis* revealed that the site of infection was the upper part of the long tubular lung. The adults penetrate into the alveolar tissue and become partially embedded in this thick portion of the

respiratory organ. The lower part of the lung, being deficient in blood vessels, is not a favorable site for the growth and development of the parasites. Only in very new infections were the adults found in the lower lung (For morphology, see Chu, 1936).

Lesions produced by the penetration of adults into the lung tissue were generally not macroscopic in light infections; in heavy infestations, hemorrhagic areas apparently caused by the penetration of the parasites in the tissue could be observed. Cyst formation as a host defense mechanism was absent in all stages examined and no evidence of toxicity was seen. Thus the relationship of *R. fuscovenosa catanensis* to the snake host is comparable to that of *R. bufonis* in the frog, for Schaake (1931) has already shown that the damage in the amphibian host is mechanical. Since no deaths of snakes in the present study could be attributed to the effects of heavy infestations under natural or experimental conditions, *R. fuscovenosa catanensis* is apparently only slightly pathogenic.

The adults of *R. fuscovenosa catanensis,* as a rule, contained only a few eggs in their uteri. In most specimens studied, from a few to 30 or 40 eggs were present. Occasionally in some actively reproducing females, as many as a hundred eggs were produced and accumulated in the uteri. These eggs were laid free in the spaces inside the lung. At the time the eggs were laid they varied from a one-celled, undeveloped egg, to a fully developed egg containing an active larva. These developmental stages were similar to those already reported for *R. bufonis* by previous workers.

The Eggs. The passage of the eggs from the lung to the digestive tract was demonstrated in many snakes by careful dissections and during this process of transference those eggs still immature continued development. Eggs were recovered in quantities often over 1,000 from the lung as well as from the feces or the rectum of infected snakes.

Though egg laying by adults in the host was probably more or less continuous this was difficult to ascertain. The passage of the eggs into the digestive tract was comparatively slow due to the fact that the elimination of the digested materials from the host was slow and irregular in comparison with the regular output of some of the ordinary laboratory animals. As the feces is not deposited and often is not even present in the rectum in enough quantity for extraction until three or four days after feeding, eggs, under these circumstances, naturally accumulated in the rectum.

As already mentioned, the eggs of *R. fuscovenosa catanensis* and of other species of *Rhabdias* hatch readily when they are in the fully developed stage. However, hatching does not seem to take place in the lung, because careful dissection of infected snakes has not shown the presence of the first stage larvae free in the lung, trachea, or upper part of the ali-

mentary tract. However, the eggs were observed to hatch immediately after contact with the physiological salt solution or water, which might easily mislead one into thinking larvae had been recovered. On the other hand the eggs often hatch in the rectum. Examination of feces from infected snakes usually showed both newly hatched larvae and eggs in various stages of development. The larvae, however, were often dead.

The process of hatching could be observed when the eggs were placed in water. Previous to the breaking of the thin shell, vigorous motions were made by the larvae inside the egg. Probably by stretching the length of the body, pressure was exerted upon the shell, which broke usually at one end. As there was no operculum or special structure in the egg shell for hatching, the position of the opening was apparently entirely a result of the mechanical strain. From the opening, usually the anterior end of the larva was first extended and slowly the whole body was freed from the shell. However, hatching with the posterior end first was observed and the process seemed equally successful. Often larvae could be seen outside the shell but still attached to it by the vitelline membrane and moving strenuously to free themselves.

The optimum temperature for hatching seemed to be about 23–25° C., most of the eggs hatching in 2 or 3 hours after recovery from the host or host feces. While some eggs could hatch at temperatures as low as 5° C. most of them were killed at that temperature.

Salt concentrations beyond physiological limits (0.85 per cent) inhibited development of the eggs and destroyed many eggs in every stage of development. On the other hand eggs in any stage of development were unaffected by 2–3 hours exposure to a 1–1000 dilution of the disinfectant metaphane.

The early larval stages: The newly hatched larvae are about 0.280 to 0.300 mm. in length and 0.016 to 0.020 mm. in width (Fig. 1). The various parts of the body are clearly visible although not well developed. The body is covered with a sheath, which is molted after the first 11 or 12 hours of growth and is only recognizable readily in the anterior end near the buccal cavity. The depth of the buccal cavity is 0.010 to 0.012 mm. The oesophagus is 0.092 to 0.100 mm. in length, with distinct divisions into the portions of corpus, pseudobulb, isthmus, and valvulated bulb. Nerve cells around isthmus are visible. The excretory pore is not visible in many specimens, but in a few the distinct paired glandular cells are seen leading to the opening, which is situated at about 0.076 mm. from the anterior end. Although the anterior portion of the intestine is quite transparent its junction with the oesophagus can be distinctly seen. Posteriorly it gradually becomes brownish apparently as food is being stored. Anus to tip of posterior end is 0.036 to 0.040 mm. The

rectum does not seem to be very well developed at this time of growth. The gonads are undifferentiated (Fig. 2).

The twenty-four hour old larvae are 0.367 to 0.483 mm. long and 0.017 to 0.020 mm. wide. The length of oesophagus is 0.110 to 0.116 mm. and the distance from the anus to the tip of posterior end is 0.050 to 0.083 mm. The gonads in the larvae of these early stages are undifferentiated and generally this development cannot be detected until the end of the second day or somewhat later. These larvae, as they feed and grow, may develop directly to the infective stage or into the free-living males and females. Those that are destined to develop directly into infective larvae may attain the following measurements at the end of 72 hours: Body length, .700 to 0.750 mm.; body width, 0.025 to 0.0333 mm.; length of oesophagus, 0.124 to .141 mm.; and anus to tip of posterior end, 0.075 to 0.083 mm.

The resistance of newly hatched larvae was tested in 1–1000 aqueous solution of the following chemicals. They were killed in the various lengths of time indicated: Iodine, instantly; Hexylresorcinol, 5 minutes; copper sulphate, 26 min.; metaphane, 3½ hours, mercurochrome, 5 hours; formalin, 10 hours.

Ordinarily newly hatched larvae feed immediately. However in the absence of food they will remain alive as long as 48 hours but will not develop, until given an opportunity of feeding. Living bacteria serve as food just as in the case of hookworm and other nematode larvae (McCoy, 1929; Lapage, 1933). This was demonstrated by the growing of larvae on pure cultures of *Salmonella schottmülleri* (*Bacillus paratyphosus* B) obtained from laboratory stock cultures[3] whereas the controls on sterile agar failed to grow. Since *S. schottmülleri* is not normally found in reptiles, and in this case came from a stock culture, it seems unlikely

[3] The writer in indebted to Dr. Calista P. Eliot for providing the pure cultures of bacteria used in these studies and for advice in the bacteriological phases of the work.

FIG. 1. Newly hatched rhabditiform larvae from an egg of the parasitic generation.

FIG. 2. Rhabditiform larva 12 hours after hatching from an egg of the parasitic generation.

FIG. 3. Infective rhabditiform larva 72 hours after hatching from an egg of the parasitic generation.

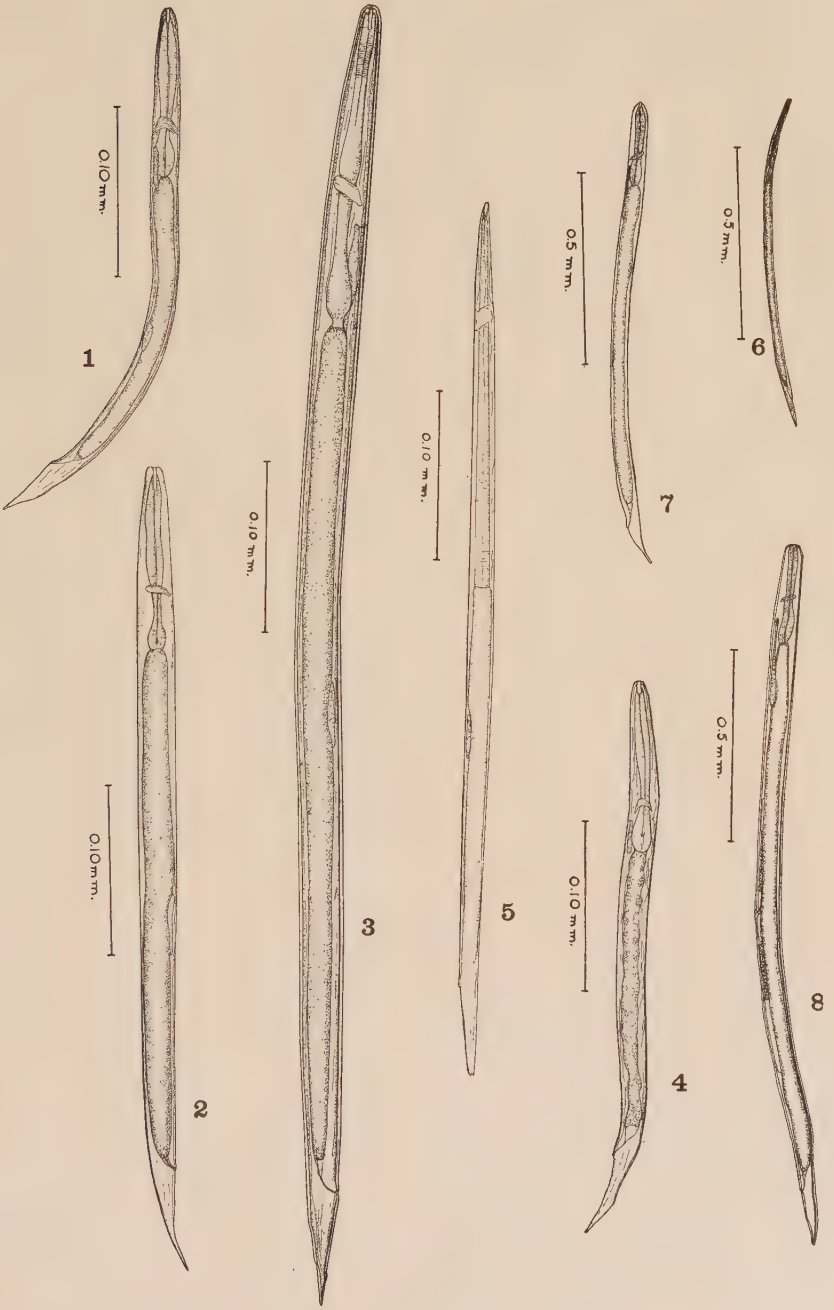
FIG. 4. Rhabditiform larva newly hatched from an egg of the free-living generation.

FIG. 5. Filariform larva, *i.e.*, "infective" larvae from the free-living generation.

FIG. 6. Developing adult, from the connective tissue between lung and intestine of *T. sirtalis*, 4 days after experimental infection.

FIG. 7. Developing adult, from lower lung of *T. sirtalis*, 10 days after experimental infection.

FIG. 8. Developing adult, from upper lung of *T. sirtalis*, 17 days after experimental infection.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that there could have been any other nutrient material transferred with them into the culture of larvae. Additional work with utilization of bacteria for food will be given later.

Infective larvae. Directly developing infective larvae first appeared in cultures at the end of 72 hours and were shown experimentally to be capable of establishing infections at this time and for at least 25 days thereafter. Immediately upon reaching the infective stage they begin migrating and are characteristically negatively geotrophic as shown by Goodey (1924). They invariably migrated over the edge of the special culture chambers and appeared in the surrounding water in the petri dish. Since, *all* the infective larvae and *only* infective larvae migrated in this manner the culture chamber served as an automatic isolation apparatus. Under favorable conditions the migration was completed at the end of the fourth day of culturing or less than 36 hours after the first infective larvae appeared. They were sheathed and distinctly rhabditiform rather than filariform and had the following measurements; Body length, 0.690–0.885 mm.; body width, 0.027–0.030 mm.; oesophagus length 0.120–0.145 mm.; distance from anus to tip of tail 0.055–0.058 mm. (Fig. 3). They remained sheathed in cultures, even after penetrating deep agar or moving actively in granular charcoal. Most of the larvae lived three weeks or longer in water but died off as the water became stale. If, however, they were washed in sterile water and then kept in clean fresh water in a shallow petri dish they lived as long as 82 days in one case. It is apparent that the infective larvae of *Rhabdias* do not feed, just as is the case with similar stages of other nematode larvae. If the water was allowed to evaporate the larvae became quiescent and appeared to be dead but revived upon the addition of water. However, complete desiccation was fatal to them.

Free living generation. In feces-charcoal cultures used in early experimental work no representatives of the free living sexual generation were encountered but only the directly devloping larvae as in the case of previous studies on this form. However, sexually mature males and females were found when the special culture chambers were first used. Since the free-living generation had never been reported for this species, despite the great amount of experimental work with it, the possibility of contamination with amphibian species was considered. In this connection mention should be made of the fact that the writer recovered viable eggs and larvae of *R. ranae* from the feces of snakes, *Thamnophis sirtalis,* fed the eggs of *R. ranae* or frogs infected with *R. ranae.* Although the possibility of similar contamination seemed remote in the previous experiment all subsequent experiments were designed to prevent even that remote possibility. Eggs were obtained (1) from the feces of snakes born and reared in the laboratory or snakes isolated for at least one month

and fed as indicated in the section on methods or (2) from the lungs of freshly killed snakes. To be certain that sufficient bacteria were present for food, *Escherichia coli,* was inoculated into all cultures. The results of culturing such eggs in the special culture chambers is given in Table 1.

TABLE 1.—*Giving the percentage total development in the special culture chambers and the actual number of infective larvae, by direct development, and the actual number of free living adults of R. fuscovenosa catanensis.*

Host	Culture number	Source of eggs	Per cent total development	Number of directly developing larvae	Number of free living adults	
					♂♂	♀♀
Thamnophis sirtalis ..	109a	Lung*	50.7	162	3	1
Thamnophis sirtalis ..	109b	Lung	19.6	46	1	0
Thamnophis sirtalis ..	109c	Feces	67.7	237	15	21
Thamnophis sirtalis ..	111a	Feces	3.6	6400	0	0
Thamnophis sirtalis ..	111b	Feces	94.9	351	1	0
Thamnophis sirtalis ..	114a	Lung	50.9	253	8	6
Thamnophis sirtalis ..	114b	Feces	56.2	658	5	0
Thamnophis sirtalis ..	116	Feces	43.2	76	11	21
Thamnophis sirtalis ..	117	Lung	50.0	1500	0	0
Thamnophis sirtalis ..	124	Lung	82.0	395	4	0
Natrix sipedon	125a	Lung	66.2	244	23	0
Natrix sipedon	125b	Lung	11.8	782	73	0
Natrix sipedon	126	Lung	39.9	119	1	0
Natrix sipedon	127	Lung	63.0	399	52	4
Natrix sipedon	130	Feces	29.0	1200	133	0
Natrix sipedon	141	Lung	50.0	285	6	8
Natrix sipedon	144a	Lung	25.0	74	2	3
Natrix sipedon	144b	Feces	53.0	180	4	0
Natrix sipedon	132a	Lung	55.0	175	1	0
Natrix sipedon	132b	Feces	62.0	532	2	1

* In cases where eggs from lungs were used, *E. coli* was added as food.

It will be seen that the direct type of development always predominated and that usually only a very few free living adults were obtained but they were obtained in all but two of the 20 experimental cultures. Of the two cultures which failed to produce the free-living adults one was started with eggs from the lungs and the other with eggs from the feces. Of the free living adults obtained, males usually predominated and could often be distinguished early in the third day by the single developing gonad. As already mentioned these adults were automatically separated from the first generation filariform larvae by the migration of the latter out of the chambers. In 72-hour cultures the following measurements were recorded: Males—length, 0.480–0.502 mm.; width 0.019–0.020 mm.; length of esophagus 0.120–0.122 mm.; distance from anus to tip of the tail, 0.040–0.042 mm. Females—length 0.672–0.685 mm.; width 0.020–0.025 mm.; length of eosophagus 0.120–0.124 mm.; anus to tip of tail, 0.039–0.043 mm. During next 24 hours the gonads developed and the females increased considerably so that the males measured only 0.516–0.546 mm. in length as compared to the females of 0.781–0.795 mm. Very little growth took place after this and the worms were sexually mature early in the fifth day (for morphology, see Chu, 1936), and mating generally occurred at this time. Copulation was frequently induced and observed throughout its process by pushing together with needles a previously isolated male

and female. The worms lay parallel and the male would clasp the female firmly by curling its tail around the female at the vulva. Once united the worms might remain in copula for as long as 5 minutes but often they separated in much less time. Both males and females would copulate several times with either the same or a different mate. Cross mating between the free living males and females of *R. fuscovnosa catanensis* and *R. ranae* could not be induced by pushing them together. That mating was essential to the production of the second generation larvae was shown by the failure to obtain the second generation from females isolated before reaching sexual maturity.

The second generation eggs and larvae. The free living females usually produce about 10 eggs most of which, unlike those of the type species *R. bufonis,* are discharged before hatching. It was observed, however, by isolating individual females that the last few eggs frequently hatch *in utero* and that those larvae feed upon the internal organs of the parent worm as do all the larvae of *R. bufonis.* These newly hatched rhabdiform larvae were quite similar to the newly hatched rhabdiform larvae of the first generation (Fig. 4).

The second generation "infective" larvae appeared in the culture on the sixth or seventh day and, when in the special culture chamber, immediately migrated over the edge into the surrounding water. These larvae measured 0.516–0.550 mm. x 0.021–0.025 mm. and were distinctly filariform (Fig. 5) as compared with the rhabditiform type of infective larvae in the first generation. The length of the aesophagus varied from 0.234–0.255 mm. and the distance from the anus to the tip of the tail from 0.049–0.052 mm. Although these larvae had the morphological characteristics and trophisms of infective larvae no infections were obtained as a result of feeding them to or injecting them subcutaneously into worm-free snakes. The propagation of the species is apparently dependent upon the directly developing larvae.

The question of strains and type of development. In order to determine whether or not there are two self perpetuating strains, one utilizing the direct and the other the indirect type of development and to see whether these types are influenced by the host as in the genus *Strongyloides* (Sandground, 1926; Faust, 1933) six different species of snakes (total of 16 animals) were infected by oral administration or subcutaneous injection of known numbers of the first generation (*i.e.,* directly developing) infective larvae, from *Natrix sipedon* or *Thamnophis sirtalis.* The percentage development was determined by autopsy of the hosts, when eggs first appeared in the feces, and eggs teased out of the lungs and adult worms were cultured. The results are shown in table 2. In every case both direct and indirect types of development occurred with the former predominating in about the same ratio as in the original cultures. In the

TABLE 2.—*Data on experimental infection of snakes with the direct development larvae of R. fuscovenosa catanensis.*

Experimental host	Method of infection	Infective larvae given		Number of worms recovered from lungs in 3–4 weeks	Per cent development in host	Number of worms developing in special culture chambers*			Per cent total development in culture
		Source	Number			Directly developing larvae	Free living adults ♂♂	Free living adults ♀♀	
Natrix sipedon	oral	*Thamnophis sirtalis*	300	30	10.0	173	1	2	55
N. sipedon	"	"	300	21	6.8	250	3	1	52
T. sirtalis	"	"	300	106	35.0	156	4	2	60
T. sirtalis	"	"	300	25	8.3	87	8	1	53
N. sipedon	"	*N. sipedon*	250	28	11.2	341	13	0	45
N. sipedon	"	"	250	18	7.4	1459	170	0	38
T. sirtalis	"	"	250	20	8.0	872	25	3	51
T. sirtalis	"	"	250	18	7.4	784	15	7	65
N. sipedon	injection	"	200	18	9.0	682	7	1	42
T. sirtalis	"	"	200	27	13.5	785	14	6	57
N. sipedon	"	*T. sirtalis*	200	17	8.5	876	4	0	48
T. sirtalis	"	"	200	22	11.0	1462	30	0	35
T. sauritus	"	"	500	88	17.6	185	1	0	70
Lampropeltis triangulum	"	"	5000	300	6.0	386	2	1	56
Coluber constrictor	oral	"	275	22	8.0	872	2	0	54
Storeria dekayi	"	"	385	29	7.5	579	1	0	75

* All eggs recovered from the lungs of the snakes at autopsy (3–4 weeks) were used in the making of these cultures.

free-living adults, males again predominated. There is no evidence that there are two strains of worms with different types of development or that the species of host effects the type of development. In order to check further, however, the possibility of strain differences all of the adults were removed from the lungs of seven snakes (4, *T. sirtalis,* 3 *N. sipedon*) and the eggs from each worm dissected out and cultured separately. Thus 1532 eggs were cultured from 85 different worms from these seven hosts and they produced 796 first generation infective larvae, 2 free living males and no free-living females. The two males came from two different worms from two different hosts and both worms produced first generation larvae, 8 and 9 each in addition to the free-living male from 21 and 19 eggs respectively. Since the eggs of a single parasitic adult have two different types of subsequent development is does not seem reasonable to expect two different strains of worms with different life histories.

Growth and Development on Pure Cultures of Bacteria. In view of the demonstration, already mentioned, that rhabditiform larvae apparently feed on bacteria just as McCoy (1929) had previously shown to be the case with hookworm rhabditiform larvae the suitability of various species of bacteria was considered. It seemed of interest in this connection to know what effect if any the type of food had upon the ratio of direct to heterogenous type of development. Accordingly the thick portion of the lung was removed under aseptic technique from a heavily infected snake, *Thamnophis sirtalis,* and teased apart in sterile physiological saline. The eggs thus washed out of the lungs were freed of debris by repeated centrifuging in sterile distilled water and decanting. On the last washing 5 cc. of fluid was left in the tube and an equal amount of 1–500 metaphane added (thus making 1–1000 dilution) and shaken to kill bacteria carried over from the lungs. Birhang (1930) and others have shown that metaphane has high germicidal powers and the writer earlier in this paper has shown that the eggs and to a less extent the rhabditiform larvae of *Rhabdias* are resistant to this substance. After 20 minutes exposure the eggs were again washed in sterile distilled water by the process of repeated centrifugation and decantation.

Known numbers of these eggs were then inoculated into 75 cc. Erhlenmeyer flasks containing 5 cc. of nutrient agar which had been diluted with 1 cc. of water. Twelve hours later pure cultures of laboratory stock of bacteria were inoculated into the flask. The delay permitted time to check the culture for sterility and prevented an overgrowth of bacteria before the eggs were all hatched. Three tests for sterility were made, viz. (1) 0.5 cc. of the fluid containing the washed eggs was inoculated into infusion broth. When such broth tubes were negative *E. coli* was later added in order to check its potential growing power. (2) A loop

from the agar in the flasks was similarly inoculated into infusion broth 10–12 hours after the introduction of the eggs and before the introduction of the organism to be used for food. (3) At the end of the experiment the organisms in the flasks were inoculated into broth and then checked by gram stain and differential culture.

In table 3 are given the results of this culturing of worms on eight different species of bacteria at room temperature (± 23° C.) and at 30° C. All eggs used were obtained from one snake, *Thamnophis sirtalis.* Just as previously, the direct type of development predominated, in fact only four cultures produced free living adults at all. Only two species of bacteria failed to support growth of the worms (*Bacillus mycoides* and *B. megatherium*) and one other species (*Staphylococcus albus*) supported growth at room temperature but failed to support it at 30° C.

A second experiment was set up using only two of the non-pathogenic bacteria (*Aerobacter aerogenes* and *Pseudomonas florescence*) which had supported growth well in the above experiment. This experiment further differed from the above in that the eggs were derived from a different species of snake (two specimens of *Natris sipedon*). Cultures were maintained only at room temperature. In some cultures dextrose or lactose was added to the culture medium and one culture with each of the bacteria was started with unsterilized eggs from the lungs. The results are given in table 4. The most interesting point in these results is the relatively large proportion of free-living adults found in the nutrient agar cultures inoculated with *Aerobacter aerogenes.* This is particularly interesting in view of the low percentage of total development in this group (about 20 per cent) as compared to the much larger percentage development recorded in tables 1 and 3. No explanation for this is available. It is an interesting but unexplained fact that neither of the bacteria used in this experiment supported the same degree of development of the worms as they did in the previous experiment (table 3). No explanation can be offered either for the low percentage of development with *A. aerogenes* on dextrose or lactose agar or on nutrient agar with unsterilized eggs.

Host infections and parasitic development. The direct development infection larvae from eggs taken from the lungs of either *Natrix sipedon* or *Thamnophis sirtalis* produced infections when fed to *Rhabdias* free snakes of two species in addition to the above, *viz., Coluber constrictor* and *Storeria dekayi* as already indicated in table 2. On the other hand repeated applications of infective larvae to the skin of snakes both on the surface of scales and between scales failed to produce infections. However, the injection of larvae subcutaneously near the lung resulted in infections in every case, four species (*Thamnophis sirtalis, T. sauritus, Natrix sipedon,* and *Lampropeltis triangulum*) being thus infected. Oral

TABLE 3.—*Growth of R. fuscovenosa catanensis larvae on pure cultures of various species of bacteria. Eggs obtained from lung of one snake (Thamnophis sirtalis).*

Bacteria	Room temperature (23° C.)				30° C.			
	Per cent total development	Number directly developing larvae	Number free living adults		Per cent total development	Number directly developing larvae	Number free living adults	
			♂♂	♀♀			♂♂	♀♀
Escherichia coli	31.6	115	0	0	3.1	12	0	0
Aerobacter aerogenes	40.2	153	0	0	10.7	41	0	0
Bacillus mycoides	0	0	0	0	0	0	0	0
Staphylococcus albus	3.7	13	0	0	0	0	0	0
Salmonella schottmülleri	53.7	200	3	1	30.2	114	1	0
Bacillus megatherium	*		0	0	0	0	0	0
Pseudomonas fluorescens	89.2	339	6	0	8.4	31	1	0
Bacillus subtiles					12.1	46	0	0

* There was a little growth, but no larvae reached the infective stage.

infections resulted in from 6.8 to 35.0 per cent and injections from 6.0 to 17.6 per cent development (table 2).

On the other hand as already noted the "infective" larvae resulting from the free living adults failed to produce infection in any of the snakes used, either from oral administration or subcutaneous injection.

Attempts were also made to infect animals other than snakes with first generation larvae. Tadpoles, snails, and cyclops were seen to actively feed upon infective larvae which had settled to the bottom of the dish. The infective larvae were also fed to the adults of two species of frogs, *Rana pipiens* and *R. clamitans;* a toad, *Bufo americanus;* and a painted turtle, *Chrysemys marginata.* There was no evidence of tissue penetration or development in any of these animals. The cyclops appeared to digest the larvae but in both the snails and tadpoles whole larvae were found in the posterior end of the digestive tract 24 hours after feeding. Many of them were evidently still alive and responded to the touch of the dissecting needle. It seems possible that snakes might at times acquire infection from eating some of the above animals shortly after the latter had ingested infective larvae of *R. fuscovenosa catanensis.* On the other hand as noted in an earlier section snakes may appear to be infected with amphibian species of *Rhabdias* as a result of eating infected amphibians.

The writer was able to confirm Goodey's (1924) report that there is an active migration of worms through the connective tissue and body cavity of snakes rather than a passive carriage in the blood stream as in *R. bufonis* in the toad (Fülleborn, 1928, and Schaake, 1931). The injection of larvae subcutaneously then does not involve any great change in the normal course of migration except for the starting point of that migration.

In light infections the larvae were usually found in the connective tissue between the intestine and lungs during the first week after injection (Fig. 6). Those measured were 0.713–0.833 mm. in length. The following week many of the larvae reached the lower lung (Fig. 7), measuring at this stage 1.97 to 2.16 mm. By the end of the third week all had reached the lung and were laying eggs. Figure 8 shows a developing adult newly arrived in the upper lung. Goodey (1924) indicates two distinct molts in the parasitic stage (third and fourth eodysis) but finds both skins still enveloping the young adult. The writer found, however, a complete molt during the first week while the worms were in connective tissue with another molt (apparently the fourth) taking place later and retained as a sheath by the mature worms (Fig. 8; see also fig. 1, Chu, 1936).

In heavy infestations a small number of worms developed at about the same rate as in light infestations. Most of the larvae, however, were

TABLE 4.—*Growth of R. fuscovenosa catanensis larvae on pure cultures of Aerobacter aerogenes and Pseudomonas fluorescens. Eggs obtained from two specimens of Natrix sipedon.*

Culture medium	*A. aerogenes*					*Ps. fluorescens*				
	Number of eggs used	Per cent total development	Number of directly developing larvae	Number of free living adults		Number of eggs used	Per cent total development	Number of directly developing larvae	Number of free living adults	
				♂♂	♀♀				♂♂	♀♀
Nutrient agar	345	17.4	39	3	8	400	33.0	130	1	1
Nutrient agar	345	22.0	56	6	14	400	20.0	80	0	0
Nutrient agar	345	24.6	78	1	6	400	18.0	72	0	0
Nutrient agar	345	19.1	53	6	7	400	11.0	43	1	0
Nutrient agar	345	12.4	69	5	8					
Dextrose agar	345	1.4	5	0	0					
Lactose agar	345	7.2	25	0	1					
Nutrient agar*	345	1.4	5	0	0	339	37.0	125	0	0

* Eggs inoculated in this case were not sterilized.

retarded in migration and development so that at the end of three weeks, when all would be sexually mature in light infestations, worms were found in every stage from practically unchanged infective larvae in the connective tissue near the intestine to the sexually mature adults in the lungs.

It is interesting to note that in spite of the active migration of the larvae there was no evidence of prenatal infection. All young snakes born of parasitized females were worm free. No attempt was made, however, to produce prenatal infection experimentally.

The duration of the infection under natural conditions has not been determined but the laboratory snakes kept under conditions designed to prevent reinfestation (see methods) were still passing eggs in the feces at the end of nine months. Apparently the infection is terminated by the discharge of the adult worm by the same route as the eggs for in a heavily infested snake (485 worms remaining at autopsy) the parasitic adults were recovered from the feces from time to time.

In view of Kreis' (1932) and Faust's (1933) report of the existence of a male in the parasitic generation of the closely related genus *Strongyloides* a careful but unsuccessful search was made for a parasitic male in *R. fuscovenosa catanensis* in all snakes autopsied. Specific experiments were also set up for this purpose. A series of small previously uninfected laboratory raised snakes were infected with several thousand larvae each and several autopsied each week thereafter for four weeks. All the organs in these small snakes were carefully searchd for worms under a dissecting microscope and the worms recovered examined under a microscope. Other snakes were given repeated infections over a period of several weeks so that they contained worms in every stage of development at autopsy. In none of the animals were any males found.

GENERAL DISCUSSION

These studies on the life history of *R. fuscovenosa catanensis* have confirmed that the parasitic adults lay their eggs in the lungs, that these eggs pass up the trachea and descend through the digestive tract in various stages of development. The writer was unable, however, to find any evidence that any eggs ever hatched before reaching the rectum and relatively few hatch there. On the other hand some eggs recovered from any point in the body nearly always hatched immediately upon coming in contact with saline or tap water. Larvae hatching in the rectum showed no evidence of development until after being passed and usually died if they were retained very long.

The concurrent direct and heterogenous development was demonstrated not only in cultures from single hosts but in eggs recovered from single worms. This is contrary to the reports in the literature which all, so far as the author is aware, indicate that one or the other only is

possible with any given species of *Rhabdias*. Previous work has indicated that only the direct type of development occurs in *R. fuscovenosa*. Furthermore the writer has demonstrated the concurrent direct and indirect development in two other species. *R. ranae* and *R. eustreptos* and Chitwood (unpublished) has confirmed it in the two later cases and found it in *R. fulleborni*. In the writer's experience with *R. fuscovenosa catanensis* and *R. eustreptos* there was an overwhelming preponderance of the direct type of development, often the indirect type occurring only as a fraction of a percent of the total development. In fact this latter type of development was not encountered at all until the special culture method giving a greater percentage total development, was utilized. Conversely the indirect type of development which alone had been previously reported for *R. ranae* was found to predominate in this species and the direct type was found only when the special culture methods were used. The writer feels that further work will probably show that both types of development occur in most if not all species of *Rhabdias* and quite naturally that the type thus far reported for a given species will be found to be the predominate type for that species. On the basis of present information one might hazard the suggestion that the direct type of development will be found to predominate among most of the reptilian species and the indirect among most of the amphibian species of *Rhabdias*. It is of interest also to note that in the case of *R. fuscovenosa catanensis* the writer was able to produce experimental infections only with the direct development larvae, *i.e.*, with the predominate type. Whether this will be universally true with *Rhabdias* or whether as in the genus *Strongyloides* both types will later be shown to be infective is open to question.

The writer does not feel that there is any suggestion in this work that environmental conditions influence the type of development as has been suggested a number of times for the genus *Strongyloides*. It seems altogether probable that what at first appears to be the effect of culture conditions upon the type of development is merely a function of the percentage of total development. Thus in the case of *R. fuscovenosa catanensis* apparently only a fraction of a per cent of the larvae are potentially able to develop into the free living generation. Therefore, any set of adverse conditions preventing development of a large portion of eggs would be quite likely to destroy all of those potentially able to develop indirectly. It is altogether possible also that the subordinated type is less resistant to unfavorable conditions than is the predominate type. Accordingly the indirect (or subordinated) type of development would appear only under those conditions which permitted a large percentage total development. The suggestion that this may not be the sole explanation is offered in one of the experiments which the extra corporeal stages were grown on pure cultures of bacteria. In cultures inoculated with

Aerobacter areogenes on nutrient agar (table 4) with an average total development of about 20 per cent over 20 per cent were of the free living generation. The writer does not feel, however, that this is other than a chance variation.

Furthermore, there was no evidence of any effect of the host upon the type of development such as Faust finds in the genus *Strongyloides.*

The report by Kries (1932) and Faust (1933) of a parasitic male in the genus *Strongyloides* stimulated a careful but unsuccessful search for a parasitic male of *R. fuscovenosa catanensis.* The fact that both types of development were found to occur among the eggs from a single parasitic worm further suggests that a functional male would be superfluous. It seems that this form should be an admirable one for a more extensive study of the cytology of reproduction in the group of *Rhabdeasoidea.*

ABSTRACT SUMMARY

The general aspects of the parasitic phases of the life history of *Rhabdias fuscovenosa catanensis* (Rizzo, 1902) have been confirmed and some details added. A new technic for culturing the free living stages is described which yields a high percentage total development. The larvae were shown to feed on living bacteria and were grown on pure cultures of several different species of bacteria. The concurrent direct and indirect type of development in the free living phases is reported, apparently for the first time in the genus *Rhabdias.* The direct type predominated and with the "infective" larvae of the predominate type only was the author able to produce infections. Infections resulted from feeding and injecting subcutaneously first generation infective larvae. Larvae applied to skin of the snake, even beneath the scales, failed to produce infections. The failure to find any evidence of a parasitic male despite a careful search is of considerable interest in view of Kries' and Faust's recent report of a parasitic male in the closely related genus *Strongyloides.* That a functional male would probably be superfluous in the genus *Rhabdias* is indicated by the fact that both the direct and indirect types of free living development were obtained from the eggs of single parasitic worms. It is suggested that these forms would be admirably suited for the study of cytology of development in the group.

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TAPEWORM STUDIES. III. SHEEP PARASITIZED WITH ONE *MONIEZIA EXPANSA* EACH*

Norman R. Stoll

While parasitization with a single tapeworm is probably to be considered a not uncommon occurrence, the recognition and study of such a condition during life is unreported for *Moniezia expansa.* In the two cases described the history of acquirement and the later findings are in agreement that in each instance one parasite was under observation. It may be postulated that the cestode under such conditions exhibits species characteristics in simple form, inasmuch as deleterious effects on the parasite coming from immune reactions of the host would be at a minimum.

In a recent article (Stoll, 1935a) attention was called to the maintenance of sheep free from *Moniezia* infection at this laboratory when kept exclusively indoors, and to an established pasture infestation in fields I and II. Infections contracted in these fields in 1933 and 1934 further emphasized the promptness with which susceptible animals secured the tapeworm after exposure there. Thus in late September, 1934, a ram in field II only 4 days became parasitized. This success led to the single day exposures herein described.

There are no indisputable clinical characteristics of a *Moniezia* infected host. The fact that the cestode infects under ordinary flock conditions means that it is usually in association with numerous nematode species the effect of whose presence confuses the tapeworm induced symptoms, if any. A host with a mature tapeworm is revealed either by the presence in the dung of eggs or proglottids, so-called segments, or both. *M. expansa* proglottids are readily visible to the eye, as whitish or yellowish-white mucus-like blobs among the fecal pellets collected directly from the rectum. The presence of the eggs is readily demonstrated in fecal specimens examined by salt flotation.

In a study of this type, comparability of day to day data should exceed even its description. This was secured with the technical assistance of Mr. P. Browne, which the author records with appreciation.

OBSERVATIONS

Case 1

A male lamb, raised on cow's milk and kept continuously indoors had been characteristically maintained free from helminths. This wether

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no. 582 (castrated at 2 weeks of age), was placed when 36 weeks old in our infested field II on Nov. 7, 1934. The day was partly cloudy, temperature ranging from 50° F. in the morning to 57° at noon. After being in pasture for 7⅓ hours, the animal was returned to its concrete floored stall. Fecal examinations were negative at 7, 8, 9, 10 and 12 days after pasture exposure. At 19 days (and occasionally thereafter) a nematode egg was observed, but no other evidence of helminthic infection was found on examinations at 31, 33, 34, 35, 36 and 37 days. Dec. 15, the 38th day, gravid proglottids of *Moniezia expansa* appeared. Daily collections were continued until Feb. 9, excepting only Feb. 3. Having shown neither segments nor eggs in the dung on Feb. 7, 8 and 9, the animal was killed Feb. 12. At autopsy a single *M. expansa* was found, a short strobila intact with scolex.

The tapeworm had thus lived in its host 97 days, for 37 of which, being immature, it failed to reveal its presence. First evidence of maturity came exactly 24 hours after the last prepatent negative examination in a twenty gram collection which contained three egg-bearing segments. The feces showed only 2 eggs per gram. Twenty-nine grams collected 2 hours later had neither segments nor eggs. Following this indication of early patency, all other specimens were positive days 38–91 after infection.

The close of the 54-day patent period was also well-marked. On Feb. 4, the 52nd day, there had been only one segment in 65 grams, the lowest proglottid yield of any positive specimen of the whole period. On the last 2 days of patency, three specimens totaling 195 grams lacked segments and had steadily lower egg counts. Feb. 5, a 9:00 A. M. specimen showed 20 eggs per gram, one at 3:30 P. M. showed 10, and at 9:00 A. M. Feb. 6 only 6. The animal was fasted 16 hours preceding the fecal collection to be made the following day. The Feb. 7 examination, however, was negative, even with the fecal concentration which followed partial fasting, nor were eggs demonstrable in the dung thereafter.

Proglottid production. Altogether 85 collections were made in the 52 patent days, Dec. 15–Feb. 4, during which segments appeared, and in 3791 grams of feces 446 proglottids were found. These collections averaged 44.6 grams with 5.2 segments each, a ratio approximately 8.5:1.0. About three-fourths of the specimens were taken in the morning. When segment yields are compared for the 15 different days in weeks I, II, III, IV and VII in which both morning and afternoon collections were made, 20 morning specimens totaling 822 grams had a segment ratio of 8.7:1.0, while 21 afternoon specimens totaled 853 grams with a ratio of 7.6:1.0. This slightly greater afternoon yield is probably not significant. During the entire period of study the sheep passed dung

in the familiar pellet form only, determinations showing 2.6 pellets per gram. The proglottids were thus present at an average rate of 1 to each 22 pellets.

Proglottids as found in freshly collected dung were usually single. Of 132 classified with respect to this point in the first patent week 91 or 69 per cent were single, 24 were in 12 pairs, 6 were in 2 groups of 3 each, and 2 strips were found containing respectively 5 and 6 segments. Of these all were adherent to the mucus on the outside of the pellets, except 7 which were caught as strips of 2 and 5 within one pellet. This unusual occurrence, illustrated in plate I, was the only time in this study when the proglottids were not seen superficial to the dung. On rare occasions in other *M. expansa* infections proglottids have been noted inside of pellets, single segments being thus incorporated least often.

While it is characteristic of *M. expansa* that segments appear singly in the dung for the most part, observations made at autopsies indicate that they are released in strips containing many proglottids. In mature infections such strips are frequently encountered post mortem in the ileum and cecum. Illustrative is a recent autopsy when 3 strips respectively 4.5 cm. long with 30 segments, 4.7 cm. long with 33 segments, and 10.5 cm. long with 68 segments were recovered from the cecum of a sheep, but all segments from the lower large intestine were single.

During the early part of the patent period proglottids were sometimes found adhering to the wool around the rump. These were usually deeply stained and flaccid, and largely devoid of eggs. Altogether 32 such segments were encountered, 20 the first patent week, 10 the second week, and after 2 on the 17th day, no others. The reason for their later absence here (as well as from ram 553) is obscure. They are not included in the determinations which follow, and are considered to have adhered to the wool under conditions when the sheep defecated without arising, a not unusual ovine habit. It has been noted also that while freshly passed dung on the surface of the bedding in a stall may show segments, it is rare indeed to find them in a similarly fresh specimen spread to any extent when defecated. The proglottids adhere more readily to dry straw, etc., than to the moist mucus coating on the pellet. This occurs also in the pasteboard containers used for taking laboratory specimens.

On several occasions during patency individual collections showed no proglottids. This occurred on days 3, 7, 8, 9, 10, 13, 14, 16, 19, 21, 28 and 48. On all except two of these days, however, proglottids occurred in from 1 to 3 other collections made within a few hours of the negative. On day 16 only one collection of 59 grams was made, and this showed no segments. On day 48 3 collections (2 A. M., 1 P. M.) totaling 138 grams were all without proglottids. Except for day 48, in

none of the first 52 patent days did two collections on the same day lack segments (day 51 not collected).

When the 54-day patent period is divided into weekly intervals, the frequency of occurrence of proglottid-negative collections shows a definitely cyclical character, as is clear from table I. In week I of patency 14 per cent of the fecal specimens showed no proglottids; in weeks II and III this rose to 31 per cent and 25 per cent, respectively, was reduced in week IV to 9 per cent, and in weeks V and VI every specimen collected had segments; week VII foreshadowed the end of the period by having a third of the collections negative for proglottids, while only 2 in the 5 specimens through day 54 in week VIII had them. Such differences are not ascribable to the size of fecal collections made, which for specimens containing or not containing segments had the same range, 20 to 85 grams, and similar average sizes, being 45.6 ± 1.2 and 45.0 ± 2.4 grams respectively.

This cyclical characteristic of the cestode's proglottid production evidenced by the entire absence of segments from certain fecal specimens is reflected in another view of the data to a certain extent independent of the above, which deals with all proglottids found in relation to the amount of feces containing them. This, it is obvious, takes into account all variation in the quantity of feces examined for proglottids from collection to collection, and is expressed on a weekly basis in table I. When stated as the number of proglottids per 100 grams of feces, a production averaging 16 in week I dropped to 6 in week II, went to 11 and 12 for weeks III and IV, rose to 17 in weeks V and VI, then fell off to 6 in week VII and 1 in week VIII.

TABLE I. *Proglottid production in successive weeks of the patent period of sheep 582 parasitized by a single M. expansa*

	Weeks of patent period							
	I	II	III	IV	V	VI	VII	VIII*
Total fecal collections	21	16	12	11	7	7	9	5
Total grams collected	919	693	463	512	400	278	421	310
Proglottids found	145	45	52	60	66	48	26	4
Proglottids per 100 grams of feces	16	6	11	12	17	17	6	1
Per cent of collections containing no proglottids ...	14	31	25	9	0	0	33	60

* Through day 54.

In text figure 1 the curve of proglottid production is shown in somewhat more detail for the entire patent period. Instead of plotting the 100 gram production of each day as found, moving averages of 3 days are used in order to indicate the trend without the more marked daily fluctuations. By this method, each day's segment yield influences three points, that for day 5, for instance, being the average of days 4, 5 and 6,

for day 6 the average of days 5, 6 and 7, etc. The graph makes clear that insofar as proglottid output in the dung of the infected host permitted visualizing the growth of *M. expansa* during its mature phase in the gut, there was shown a high production of segments very early after the cestode reached maturity, which dropped off strikingly by the second week, increased gradually to another period of high productivity, then again a marked falling off, to entire failure of production. The recurrence of minor cycles throughout the curve may also have biological significance.

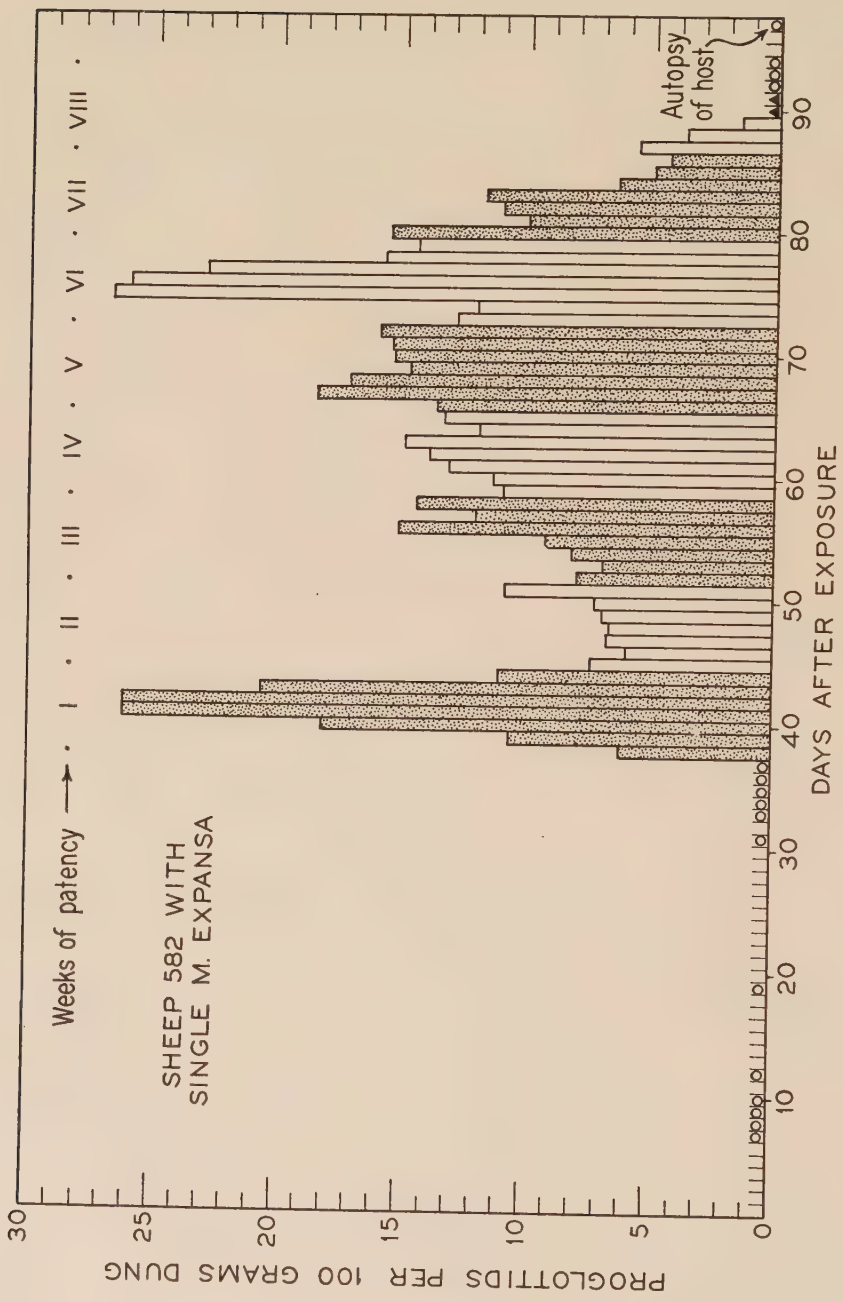
Egg production. Freshly secured proglottids were examined for their egg content. On the 4th patent day two fresh segments, still attached, crushed in water showed 23,200 eggs, or 11,600 each.

It being noted that in the interval before these were crushed many eggs had been extruded into the water, on the 6th patent day 19 segments were initially left undisturbed in water for 50 minutes and then transferred to a second container for crushing. The extruded eggs totaled 110,000 (see plate I), and after crushing 98,000 more were recovered. These 19 segments averaged 10,947 each for egg content, of which 53 per cent were set free into the water by the action of the proglottids themselves.

On the 7th patent day 13 segments were placed in a dish in a humid chamber only, no water being added to the proglottids directly. After two hours the segments were carefully removed and after rinsing in water in a second container, were transferred to a third for crushing. The three lots of eggs were then counted separately. It was found that these 13 segments had extruded 99,000 eggs under merely moist conditions; 16,000 additional were washed into the rinse water, and 86,000 set free by crushing. The total of 201,000 eggs averaged 15,462 per segment, of which 49 per cent were set free by the proglottids in the moist chamber, or, with the rinsed eggs added, 57 per cent of the total yield. Counts of the egg supply contained in proglottids from other *M. expansa* infected animals indicate that yields in excess of 15,000 per segment are frequent, and not uncommonly exceed 20,000.

The determinations on the three batches of proglottids above, at 11,600, 10,947 and 15,462 average a segment count of 12,670 eggs. The fecal specimens from which the proglottids were removed were not all separately counted, but even disregarding the eggs which escaped from the segments onto feces in the 10–15 minutes before removal, there is indicated for the first patent week a minimum egg production of 9,223,760 eggs, based on an estimated 728 segments voided, or 1,317,680 eggs daily for the one tapeworm.

The extent to which the observed egg production of the first week was maintained throughout the patent period, was not determined.


PROGLOTTIDS PER 100 GRAMS DUNG
0
5
10
15
20
25
30
DAYS AFTER EXPOSURE
10
20
30
40
50
60
70
80
90
Autopsy of host
Weeks of patency
I
II
III
IV
V
VI
VII
VIII
SHEEP 582 WITH
SINGLE M. EXPANSA

although certain data suggest it may have diminished but little on a proglottid basis. Thus, on the 8th patent day, 5 proglottids extruded 61,800 eggs into a dish of water in 3 hours, and 22,600 more were counted after crushing the segments, an average production of 17,680. One of 13 segments collected on the 39th patent day was egg counted after having been refrigerated 9 months and showed 13,200 eggs. In early July, 1935, 138 refrigerated segments collected on 20 of the days between Dec. 20 and Jan. 27 (days 6 to 44 of the patent period) were crushed and pooled, showing 940,000 eggs, or 6812 per segment. As some of the proglottid tissue had become matted due to mold growth in the dishes and thus by partial failure to disintegrate did not permit the material to be well-counted by dilution, this figure is obviously lower than the actual.

We are not yet certain whether the extrusion of the eggs from the proglottid is due to the fact that segments fresh from the rectum are still alive, as is true in some cestode species, or whether the occasional movement noted in the segments just placed in water is due to muscular contraction caused by inbibition of fluid. If alive when voided, the proglottids die in a few minutes, although under favorable conditions the eggs appear to stream from slight knob-like protrusions on the margin of the proglottid for perhaps an hour or more. This extrusion of the eggs can be seen to take place onto the dung surface itself if the segment is kept moist and unchilled, and will make a distinctly white spot of eggs against the greenish-black, somewhat shiny, background of the pellet. Hall (1934) has described an apparently analogous "discharge of eggs" from segments of *Thysanosoma actinoides,* another anoplocephalid of sheep.

Reference has been made to proglottids found early in the patent period on the wool of the rump, and of very low content of eggs. Thus on the 8th patent day, when 5 segments just voided showed 88,400 eggs, or 17,680 each, 4 segments collected on the same occasion from the rump wool showed a total of only 4600 eggs, which at 1150 each, was 93 per cent less. At the time it was assumed that this represented evidence of eggs deposited on the wool of the rump, which can, in fact, be readily demonstrated.

TEXT FIGURE 1. Segment production of a single *Moniezia expansa* in sheep 582, plotted as 3-day moving averages of the number of proglottids per 100 grams of dung, which appeared through a 52-day period (followed by 2 days when eggs only were demonstrable). Circles indicate negative examinations in the prepatent and postpatent periods. Grazed in infested pasture Nov. 7, 1934, only, the animal had a prepatent period of 37 days, was patent from 38th–91st days, and killed on the 97th day, had one short strobila, intact with scolex. The patent period is labeled by weeks in Roman numerals with alternate weeks stippled in the bar diagram.

Recently while studying another infected animal an alternative explanation developed. When several collections were made at intervals of a few moments, and the proglottids separately tubed in water within the minute, seriatim, it was found that segments in the initial sample frequently had very low egg counts, representing 5 per cent or less of the counts of individual segments obtained only a few minutes later. Rarely would an excessively low count segment appear in the latter part of the series. The reason for this is apparently clear: pellets and segments of the initial specimen may have been held in the rectum for some time preliminary to normal defecation, whereas specimens secured after an interval probably represent dung which had not yet entered the rectum when the initial specimen was taken. As the late proglottids have consistently higher egg counts, there is evidently extrusion of many eggs on to the feces in the rectum when segments remain there for a period. This would harmonize with a recent observation by Honess (1935) that pellets from the colon of a rabbit harboring mature *Cittotaenia variabilis* show numerous eggs on their surfaces, most of which can be removed by simple washing of the pellets.

The loss of eggs from segments in the rectum of either sheep or rabbit is likely due to mechanical factors present in a relatively large mass of contiguous pellets as compared to the single row of pellets in the large gut itself first in the making and then in continual forced movement posteriad by peristalsis. Also this is probably greater in degree for the softer proglottids of *M. expansa* in sheep than for the firmer, more heavily muscled segments of *C. variabilis* in the rabbit. It would even appear that some *M. expansa* eggs are mixed with the fecal material well anterior to the rectum, as evidenced by the occurrence already noted of a pellet molded about two groups of segments, a condition that must have forced the extrusion of some, perhaps many, eggs into the fecal substance.

Two other observations in our study of sheep 582 suggest a similar point. On the 16th patent day a 59 gram specimen contained no proglottids but had a count of 3600 eggs per gram, or 212,400 for the specimen. As this constituted about 1/11th of the computed daily fecal output, a day's egg production of over 2,300,000 is indicated. This, it will be noted, is somewhat higher than the minimum egg production estimated for week I of the patent period on the basis of proglottid egg counts. The entire absence of segments in this specimen may mean that some were in the rectum and failed to be evacuated with the specimen, although we have no observations to indicate that if present there they fail to appear at either a natural or induced defecation. Its more likely meaning to us is that conditions favorable to the event produced a large extrusion of eggs from passing segments into the intestinal debris higher

up in the gut. Again at the termination of the patent period, while the last segment was observed on the 52nd day, eggs in decreasing numbers consistent with absence of segments, continued 48 hours longer, a period possibly permitting emptying the cecal contents of the 52nd day.

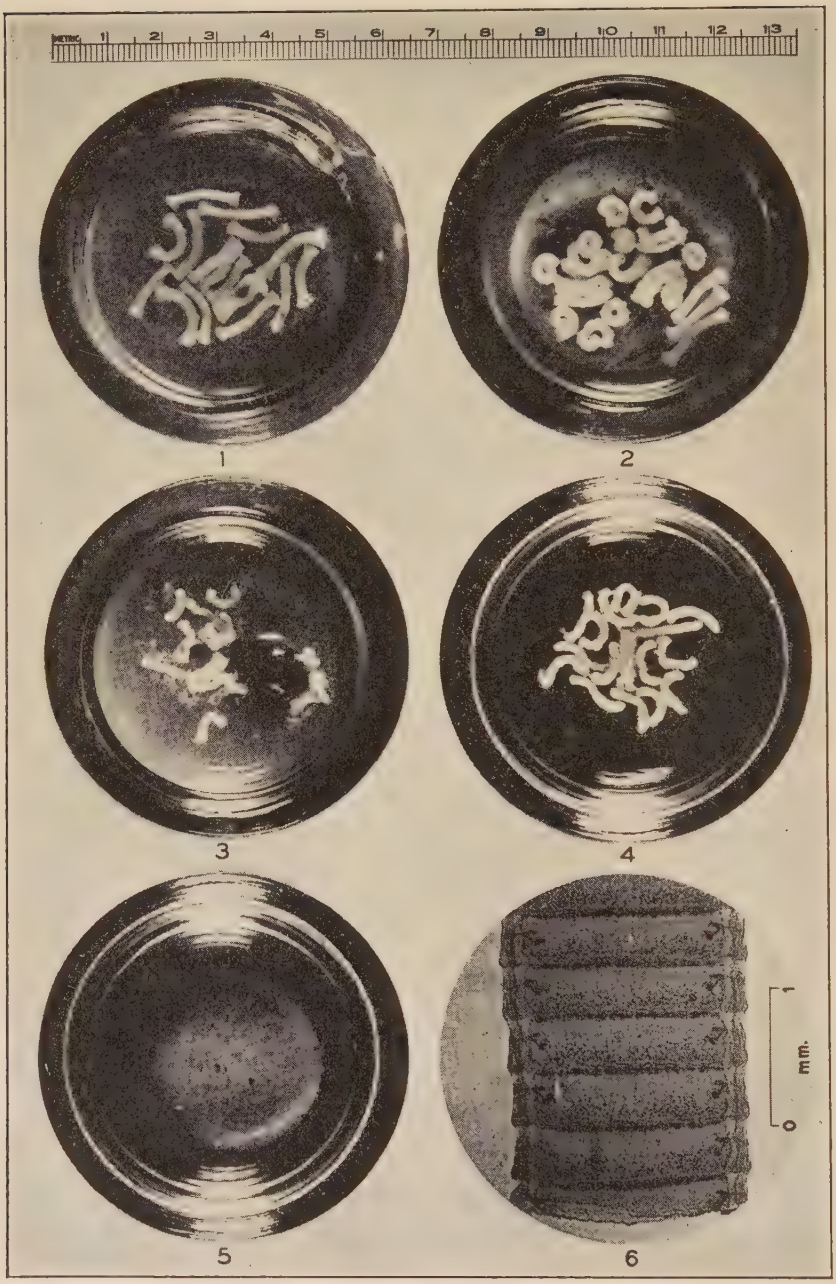
Condition of embryos. Throughout the course of infection eggs crushed for examination indicated the presence of vigorous hexacanth embryos. The last observed were of the 50th patent day. Appearances of eggs throughout the ensuing 4 days suggested no modification.

Proglottid size. Measurements were made of the breadth of many of the segments when placing them in water right after collection. Their average and range in mm. for different weeks of the patent period were: I 12 (8–14), II (not measured), III 9 (6–12), IV 10 (6–15), V 7 (6–9), VI 6 (4–10), VII 6 (5–6), VIII 7 (6–7). These may be accepted as close to "natural" size, in view of the fact that several of them, after being plumped in water, fixed in formalin, stained in haematoxylin and mounted in balsam without coverslip pressure, show close to the same sizes. Thus 9 all originally measured as 6 mm., after mounting averaged 5.8 mm. (range 5.5–6.0) ; 3 others originally measured as 7 mm., after mounting were respectively 5, 6 and 7 mm.

The antero-postero length of the mounted segments averaged 1.5 mm. (range 1–2).

Segments when placed in water not only extrude eggs but become enlarged, as is shown in plate I. Those of the 4th patent day when photographed had been in water 48 hours, of the 5th day 24 hours, of the 6th day 2 minutes. The segments of the 6th day are shown again after being in water 61 minutes longer, an accompanying dish displaying the egg supply (totaling 110,000 by later count) which had been extruded in the first 50 minutes. The increased segment size in the hour's additional water exposure and consequent plumping is clear. The knob-like protrusions of "egg laying" proglottids are obvious in two of the segment groups.

Size of strobila. The strobila with intact scolex recovered at autopsy 97 days after exposure and 8 days since the last segment had been observed was fixed in 5 per cent formalin, haematoxylin stained, and mounted in balsam without coverslip pressure. Its total length was then 14 cm. and width at posterior end 1.8 mm. The scolex, 0.69 mm. in diameter, lacks hooks, of course, and has the four suckers characteristic of *Moniezia* spp., two of them measuring respectively 0.24 mm. and 0.25 mm. in diameter. The head is about equal in breadth and length, the neck narrowing to 0.31 mm. Segmentation is first visible 2.1 mm. posterior to the scolex, and the discrete interproglottidal glands of the *expansa* type first appear about 6 cm. further posteriad, where the strobila is 1.4 mm. wide. The testes are well defined, as are other beginnings of


1
2
3
4
5
6
mm.

the genital organs, but the absence of complete development of the reproductive system indicates that portions of the strobila, probably several centimeters in length, had been lost by the worm in the 8-day interval since the last proglottid had been observed by us, this last having been egg bearing.

Fig. 6 in plate I shows the posterior portion of the strobila within 1 cm. of its termination.

Perspective of cestode growth. In an earlier article (Stoll, 1929) it was shown that sheep studied here have exhibited a fairly constant relation between body weight and average daily grams of pellet output. Sheep 582 weighed 65 pounds on the 20th patent day, a weight maintained without marked gain or loss until its slaughter 40 days later, as shown by reweighing at autopsy. A mean fecal output of 650 grams daily is thus indicated.

On the basis of a feces: proglottid ratio of 8.5: 1.0 the tapeworm thus matured 3976 segments during days 1–52 of the patent period. This was an average daily rate of 76 segments, and reached over 100 daily in weeks I, V and VI.

The 1.5 mm. mean length of such segments determines a total linear growth of mature proglottids during days 1–52 of nearly 6 meters (596.4 cm.) or 11.5 cm. daily, reaching 15 cm. daily in weeks I, V and VI.

The several observations on freshly examined segments indicate a mature proglottid egg content ranging between 10 and 20 thousand. For 3976 segments this would place the total egg production of this tapeworm in the range of 40 to 80 million eggs for the entire patent period, approximately 1 to 1½ million eggs daily.

The fact that the anterior portion of the cestode was found in good condition at autopsy 8 days after the last observed proglottid, may have meant that the organism was passing through a period of recuperation antecedent to a new cycle of egg production, as observed later in the parasitism of ram 553. Such a stage would be comparable to the char-

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PLATE 1. Illustrating *Moniezia expansa* proglottids collected during the first patent week from the single parasitism of sheep 582. The scale, in millimeters, applies 1–5 only.

1. Segments of 4th patent day after being in water 48 hours.
2. Segments of 5th patent day after being in water 24 hours.
3. Segments of 6th patent day after being in water 2 minutes. An unusual condition of one pellet containing 7 segments in shown.
4. Segments of 6th patent day after being in water 63 minutes. The pellet was removed, its segments separated.
5. Eggs (totaling 110,000 by later count) extruded from the 19 segments illustrated in (3) and (4), within 50 minutes after placing in water.
5. Proglottids 1.8 mm. wide from the terminal centimeter of the *M. expansa* specimen recovered from sheep 582 at autopsy. Haematoxylin stain.

acteristic new growth of the strobila following retention of the scolex after administration of an anthelmintic, which is usual rather than unusual in cases treated for tapeworms. Africa and Garcia (1935) appear to have such a demonstration with the rare human anoplocephalid, *Bertiella* sp. So far in our sheep experience wether 582 is an exception in showing *M. expansa* at autopsy after recently becoming negative by fecal examination. Two sheep under observation in the 1934 season illustrate the usual condition. Lamb 600, *M. expansa* positive for some time up to Sept. 6, died two days later and was negative at autopsy for both worms and eggs. Lamb 599, similarly positive for several weeks up to Dec. 7, when killed 4 days later following intracerebral inoculation with an encephalitis virus, was negative at autopsy for both worms and eggs.

The situation raises the question as to whether no. 582 may not have become reinfected with a single egg in its stall during January a few weeks before slaughter. The stall had been cleaned weekly. While such speculation would not necessarily impair the reported record of no. 582 as a case of single infection with *M. expansa,* in the light of observations on ram 553 which follow it seems necessary to regard the tapeworm found at autopsy as the one under observation throughout the patent period, rather than as a new accidental infection.

Supplementary items. Blood slides of wether 582 were made on Jan. 3 and before autopsy, Feb. 12, respectively 57 and 97 days after exposure to infection. On the former date, the 20th patent day, eosinophiles were 2 per cent; on the latter, the 6th post-patent day, no eosinophiles were seen.

Post mortem the only helminths found besides the tapeworm were one adult female each of *Chabertia ovina* and *Oesophagostomum columbianum* from the large gut. In the wall of the abomasum were a few lesions characteristic of *Trichostrongylus axei.* Nematode eggs in small numbers (1 to 4 per gram) had been noted in fecal examinations beginning Nov. 26, a week after the last preceding negative, and from appearances were ascribable to the *Trichostrongylus.*

Except for scattered coccidial lesions in the small gut, there was no pathology. The excellent condition of the organs at autopsy reflected the persistent normal health maintained by the animal throughout the tapeworm infection. The failure to gain weight, already noted for the period Jan. 3 to Feb. 12, is not unusual for uninfected indoor animals that time of year.

Case 2

We have under a tree in our infested field II a nine-foot square enclosure into which in the fall of 1933 sheep dung containing many

millions of *M. expansa* eggs was spread. The enclosure is in a depressed area between two slopes of the pasture, and except for the lower boards of its fence panels would be readily subject to wash from surrounding land under conditions of heavy rain.

Ram 553, born Nov. 30, 1933, bottle raised on cow's milk, and used in an *Haemonchus* experiment, had always been kept indoors. On Dec. 28, 1934, after being fasted 18 hours, this yearling ram was placed in the field II enclosure, in which at the time there was no sign of the fecal material placed there nearly a year and a half earlier. Dec. 28 was crisp and clear with a temperature range from 34° to 43° F. following a night on which the thermometer had dropped to 17° F. leaving the ground surface frozen. In the enclosure there was abundant long dry grass of an undisturbed season's growth, which the ram was observed to feed on during his stay there from 10:00 A. M. to 4:30 P. M. He was returned to a concrete floored stall after these 6½ hours.

Twelve days later, on Jan. 9, the dead grass having been raked from the enclosure to expose better a short green growth underneath, ram 553 was again placed there after a day's fast. The day was foggy, temperature ranging from 50° F. in the morning to 58° at noon. Further to induce the animal to get close contact with the somewhat softened soil, oats which he was later observed to eat were scattered on the ground. After 8 hours he was again returned to his stall.

It had been planned to expose this animal a third time about 2 weeks later, but snow covering the ground made it inexpedient. On Feb. 6, however, he was fed the dry grass which had been raked on Jan. 9 and kept in a sterilized bag since. Although the grass had partly molded, the ram was induced to eat most of it.

No insects had been observed in the enclosure, despite search, on either Dec. 28 or Jan. 9, nor in the dry grass fed on Feb. 6.

On Jan. 10 and 18, and at 2-day intervals Jan. 31 to Feb. 14, fecal examinations of the animal were negative except for a low residual Haemonchus count. Nine days later on Feb. 23, *M. expansa* eggs were found. Beginning Mar. 1, proglottids were present, and for 15 consecutive examinations spaced 1 to 4 days apart through Apr. 5, the feces were always positive, only two of the specimens lacking segments. On Apr. 9 and 12 neither proglottids nor eggs were demonstrable, but on Apr. 16 eggs were again found and in examinations on 9 days before the animal was killed on May 6, segments were invariably present. At autopsy one *M. expansa* was found.

It is clear that the original infection could not have occurred from eating the dry grass fed on Feb. 6, for the animal was known to be positive 17 days later on Feb. 23. As the last prepatent negative and the first recognized patent positive were respectively 48 and 57 days

after Dec. 28 and only 36 and 45 days after Jan. 9, it is equally clear that infection took place on the latter date. Assuming a 37 day prepatent period similar to that of wether 582, the examinations of ram 553 which were positive for proglottids, occurred on the following days of the patent period: 14, 15, 16, 18, 20, 22, 24, 25, 32, 35, 42, 46, 49, 63, 67, 70, 74, 76, 77, 78, 79 and 80. On 4 additional days, 8, 28, 39 and 60, the feces contained eggs but no segments, while on days 53 and 56 neither proglottids nor eggs were present. The proglottids present on days 76–80 were not counted. On the other 21 positive days 1684 grams of pellets contained 51 segments giving a feces: segment ratio of 33: 1. On 21 days the pellets averaged 1.7 per gram, producing a pellets: segment ratio of 56: 1.

The fact that following a positive specimen on Apr. 5, the 49th patent day, neither segments nor eggs could be found 4 and 7 days later but reappeared at 11 days and thereafter suggests that we were in reality observing two successive periods of mature segment production separated by an inter-patent period, in which the cestode may have been much reduced in size, as was found for the specimen in wether 582 at autopsy. This inter-patent period may have had a maximum span of 10 days (Apr. 6–15). Seddon (1931) cites an infection suggestive of a parallel to ram 553. His lamb no. 700 was exposed 11 days in an infested pen, was negative 35 days after the first day of exposure and *Moniezia* positive at 41 days, which was the next examination. "The total period covering positive faecal examinations covered 63 days. There were, however, two distinct periods of egg-laying with a fortnight in between during which no ova could be detected." He further observes "it would possibly appear, therefore, that this animal had two distinct infestations, but the second following closely on the first." The absence of further details does not permit determination of whether such reinfection could be dated back to the original exposure period, or whether the data would better fit our observations on ram 553.

The comparative rate of segment production before and after the negative period in ram 553 further suggests a double cycle of mature proglottid production. When the segment production is compared for successive weeks of the initial patent period (assumed to have begun the 38th day after exposure to infection on Jan. 9th), and is expressed in terms of segments per 100 grams of feces, the rate is as follows: week I (no examinations), II 2, III 3, IV 2, V 3, VI 1, VII 2, VIII 0, IX 8, X 7, XI 5, XII (segments not counted). The production of proglottids is thus shown to have been at a relatively constant rate of 1 to 3 per 100 grams in weeks II–VII inclusive, but at the renewed patency two weeks later was much higher, with another tendency toward reduction within the period of study.

While the average proglottid production for the cestode in ram 553 during weeks II–VII of the patent period was only 2.2 per 100 grams feces, compared to 11.5 for the same period of the tapeworm in wether 582, this disproportion needs correction for weight of animal. Ram 553 weighed 102 pounds on Jan. 18 and gained at the rate of over a pound a week until slaughter on May 6. If his average weight of 110 pounds is used to estimate the fecal output, this becomes 1100 grams daily. This difference in fecal output is indirectly shown in the larger size of pellets from ram 553, and the larger amounts of dung at routine collections, which averaged 76 grams as against 45 grams for no. 582. On a comparative daily basis during patent weeks II–VII only, average proglottid production from wether 582 was 75, while from ram 553 it was 24. In the period following the interpatent negative phase in the latter animal this was, however, increased to an average of 73 daily, over at least a 15-day period. Moreover, a 48-hour fecal collection of ram 553 in the last week of its life showed an *M. expansa* egg count of 1,570,000 daily. These data indicate that during at least a part of the period of their lives the reproductive activity of the two tapeworms concerned was of the same magnitude.

The autopsy of ram 553 on May 6 was 117 days after exposure to infection on Jan. 9, and 80 days after the assumed prepatent period of 37 days. The tapeworm found was measured in water without stretching after refrigeration over night. This procedure gives plump worms whose measurements do not change markedly even after formalin fixation, and has been used as a standard to compare with balsam mounts later.

The cestode was broken once during its recovery so that as measured it represented an anterior portion with head, 230 cm. long and 12 mm. wide posteriorly, and the detached portion of strobila 89 cm. long and of approximately uniform 12 mm. width, a total length of 319 cm. There were 12 segments in the last 2 cm., giving an antero-postero proglottid length of 1.7 mm. each. These proglottids contained eggs whose embryos would activate on pressure. In the cecum were approximately 7 cm. additional proglottids, including several in single form as well as attached groups of 2, 4, 12 and 16. Characteristic discrete interproglottidal glands of the *expansa* type were demonstrable.

The question of whether the period of renewed patency may have arisen from the feeding on Feb. 6 of dry grass from the infested area seems to require a definitely negative answer. This secondary patency was observed Apr. 16, following negative specimens on April 9 and 12. The former date is 69 days after Feb. 6, practically twice as great a prepatent interval as is established for *M. expansa.* Moreover what grass was eaten by the ram in the enclosure itself on Dec. 28 proved uninfective for him, although the closer contact with the soil 12 days

later did prove infective. The fact that grass raked from the area on Jan. 9 molded by Feb. 6 when fed, may have militated against getting an infection by this method. However, fresh spring grass pulled from the same enclosure later on March 29, kept fresh in water over night and fed to a sheep (then susceptible as proven by ability to infect the animal in a later experiment) on March 30 failed to produce infection, nor could any eggs be demonstrated in the water which had held this grass.

The only other source of reinfection for ram 553 was his own stall. On March 1 the ram was placed in a double stall alone, and contrary to usual procedure, the stall was deliberately left without weekly cleaning. Straw, occasionally added to the bedding, produced an accumulation of several inches of manure in the 9 weeks from early March to early May. To have become infected here so as to show a negative fecal examination April 12 and a positive April 16, would require contraction of new infective material between March 6 and 13 (Stoll, 1935 b). When a control experiment was later set up with a susceptible and a *M. expansa* infected sheep in a stall left without weekly cleaning in the same way, no infection resulted, although *Moniezia* eggs could be demonstrated in transit through the susceptible animal, which did obtain infection promptly on infestive pasture later.

Supplementary observations. During the series of fecal examinations on ram 553, besides the *M. expansa* eggs and a low *Haemonchus* count, there was observed on April 9th eggs of *Trichuris ovis.* This date was 90 days after Jan. 9th, when it is assumed the infection was contracted. The eggs, in the 1 cell stage, were noted also on Apr. 16, 30 and May 3, 4 and 5. The examination technique used would not necessarily have demonstrated *Trichuris* eggs when they first appeared, nor would they *Capillaria* in small numbers.

At autopsy besides *Haemonchus* in the abomasum and *M. expansa* in the small gut, there were found two *Capillaria longipes,* a male 13.3 mm. long and a female 15.8 mm. long, as measured in glycerin-alcohol later. The female had no eggs, but whether due to immaturity or senescence is not known. In addition in the cecum there was one female *T. ovis* containing eggs. Thorough search failed to reveal any male whipworm, although it is possible one may have been present earlier.

Occasional blood examinations on ram 553 had shown an eosinophilia, but this pre-dated the *Moniezia* exposure and is referable to the earlier *Haemonchus* experiment. As noted, throughout the period of the *Moniezia* infection the ram gained weight and was in excellent health, as was reflected by the condition of the organs at autopsy.

DISCUSSION

In the present state of knowledge, identification of a given *M. expansa* infection during its life as being due to a single tapeworm must needs take into account such factors as demonstrable absence of exposure of the host preceding the period of study, limited exposure to produce the infection, and subsequent history. Inherently difficult as such conditions are in the problem, the two infections described appear to satisfy the requirements, with the worms recovered at autopsy representing the solitary cestodes responsible for the manifestations of the earlier patent periods. The alternative, barring contamination of a sort scrutiny has failed to reveal in dozens of animals kept under observation indoors, is to believe that each animal, while still supposedly non-immune, respectively reinfected himself indoors in midwinter with one tapeworm each, a coincidence rather too far-fetched to entertain seriously.

Wether 582, it will be recalled, was infected in 7⅓ hours' exposure on Nov. 7 in a 3.5 acre field. Ram 553 was infected in 8 hours' exposure on Jan. 9 in an area of 81 square feet. It is of interest to note the conditions under which two other single *Moniezia* infections, discovered as such at autopsy, were contracted. In Nov., 1926, Smith and Ring (1927) found one mature *M. expansa* in a young ram, bottle raised, pastured in field IV, an area which had not hitherto been used for sheep. The source of this infection is undetermined (Stoll, 1935a), and the ram contracting this single tapeworm was the only one of 8 animals similarly handled that surely became infected. The animals were thus shown to have been exposed to a minimal infestation, which is explainable in case the infective stage of *M. expansa* could contaminate the footgear of caretakers. It happens that the men who had these animals in their care in field IV from May to Nov. 1926 were under the necessity of traversing the lower edge of field V to reach them for daily watering, etc. Field V became infested with the eggs of *M. expansa* by an infected sheep in Nov., 1924, and a susceptible animal in field V during 1925 may also have had the infection, which the fecal examination schedule of Smith and Ring missed.

Seddon (1931) reports a lamb, no. 581, with a single *M. expansa*. It was exposed with 3 other lambs, another of which was also susceptible and one "affected with *Moniezia*" in his Pen E, "actually a gravelled pen slightly sloping and devoid of vegetation." Lamb 581 was last negative 50 days after first exposure, and was positive at 57 days which was the next examination. Killed on the 88th day it had one Moniezia. While Seddon believed "previous faecal examinations would indicate that the infestation had earlier been much heavier," the "bare ground" conditions under which the infection was contracted and the lag period

after exposure, as well as the fact that a second susceptible lamb (no. 574) exposed with this animal failed to become infected (70 days) suggests instead that the autopsy evidence of a single *Moniezia* reflected the true condition of the parasitism. Killed 31 days after the first known positive examination (although it may have been positive up to 6 days longer) the decrease by then in the number of eggs in the dung falls in well with our observations on no. 582 and no. 553.

The curve of proglottid production in the case of no. 582 would appear to indicate a definite rhythm of growth or of reproductive activity in this cestode. However, the extreme effects in weeks I and VI of patency may have separate explanations. Wether 582 became patent on the 38th day, which is 2–3 days longer than has been required for several of our animals. This may mean that some delay attended the initial release of gravid proglottids, following which more than the usual number of segments was discharged in a period of a few days. This over-compensation may have reduced the supply to the strikingly subaverage condition in week II, and part of week III. The plateau in text figure 1, which extends from the middle of week III into week VI, is striking. The second period of high proglottid output may in turn have been premonitory of an approaching senescence or lethargus in this particular tapeworm. Perhaps even, the parasite may have begun to feel the effects of a beginning immune state in the host.

Interpretations hinge, of course, in part, on the degree to which the appearance of segments in the dung, either per 100 grams of feces or on the estimated daily output, reflects what is actually occurring in the life of the cestode. It is conceivable that irregular holdup of segments somewhere between the place where they are lost from the worm and finally voided by the sheep underlies the cycles. It is also possible that proglottids are always produced at an approximately even rate, and the ups and downs of segment appearance externally are due to unmeasured changes in the peristalsis of the host. At present these hypotheses seem improbable as the explanation of the observed phenomena.

SUMMARY

1. Two sheep, each harboring one *Moniezia expansa* have been studied primarily with reference to the development and maintenance of the cestode infections.

2. Parasitism resulted from exposure to infested ground for $7\frac{1}{3}$ hours in November and 8 hours in January respectively.

3. In the first case a prepatent period of 37 days was followed by a patent period of 54 days, although proglottids had ceased appearing two days earlier. Six days after the end of the patent period a single speci-

men of *M. expansa,* intact with scolex but not showing mature segments, was recovered at the autopsy of the host.

4. A definite cycle of proglottid production was observed. It was high at the beginning of patency, then dropped sharply and gradually rose again to about the original level, before finally tapering off.

5. The basis is given for the estimate of a total production during the patent period of about 4000 proglottids and between 40 and 80 million eggs. Mature segments totaling about 6 meters were discharged.

6. In the second case, not followed in such detail as the first, there was indicated a similar prepatent period, followed by a patent period of 7 weeks, an interpatent period of a week and a period of renewed patency until slaughter of the host 4 weeks later when one mature *M. expansa* was found.

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FURTHER REPORT OF A STUDY ON THE LIFE CYCLE OF *GNATHOSTOMA SPINIGERUM**

CHALERM PROMMAS AND SVASTI DAENGSVANG

Since we reported in 1933 the finding of the development of *Gnathostoma spinigerum* larvae in the body cavity of the cyclop (Prommas and Daengsvang, 1933), in the belief that probably the parasite has only one intermediate host, attempts were made to infect cats with *G. spinigerum* larvae in cyclops by feeding but with negative result. Thus our attention was directed to search for animals which possibly may act as its second intermediate host. These experiments on cats and the second intermediate host are described in this paper.

FEEDING EXPERIMENT ON CATS

The cats used in the experiment were not laboratory bred but were bought from local dealers. They were divided into 3 groups, group I contained 10 cats, group II, 10 and group III, 8. The animals of group I were kept in the laboratory for a short period before the experiment and proved negative for *G. spinigerum* by fecal examination on 3 successive days while those of group II and III were kept at least 6 months and subjected to frequent stool examinations and all of them failed to show ova of *G. spinigerum* in their feces.

The cats of these three groups were fed with various numbers of cyclops containing *G. spinigerum* larvae of different ages and numbers (see table I, II and III). Although the larvae fed were of different ages, they all had attained similar morphology. They showed the following structural characters: a cephalic bulb with four transverse rows of single-pointed spines and a pair of lips; the cuticle with transverse striations throughout and for about the anterior fourth of the body covered with transverse rows of minute rudimentary single-pointed spines; the presence of two pairs of transparent contractile cervical sacs; an alimentary system consisting of a buccal cavity, a strong muscular esophagus and an intestine with a subterminal anus.

The cats of group I were fed by a stomach tube under chloroform and those of group II under avertin narcosis. In group III the cyclops were given by natural oral feeding without the help of narcosis or a stomach tube. The experimental cats were killed by anaesthesia or died from other causes at various periods after feeding and then were

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subjected to careful post-mortem examination. The results of the experiments are shown in tables I, II and III.

TABLE 1.—*Result of Feeding Experiment on Group I*

Cat. No.	No. of *G. spinigerum* larvae in cyclops	Age in days of *G. spinigerum* larvae in cyclops	No. of days after feeding	Autopsy findings	Remarks
1	41	7	30	Negative for *G. spinigerum*	Killed
2	43	7, 8	97	" " " "	"
3	41	8	48	" " " "	Died
4	46	8, 9	30	" " " "	Killed
5	40	8, 9	35	" " " "	Died
6	44	8, 9	71	One immature female of *G. spinigerum* in the stomach wall and one mature male in the peritoneum	Killed
7	44	9	30	Negative for *G. spinigerum*	"
8	30	8, 9, 10	91	One immature female of *G. spinigerum* in the right side of diaphragm	"
9	..	..	..	Negative (control)	Died 32 days after experiment
10	..	..	..	Negative (control)	Killed 101 days after experiment

TABLE 2.—*Result of Feeding Experiment on Group II*

Cat No.	No. of *G. spinigerum* larvae in cyclops	Age in days of *G. spinigerum* larvae in cyclops	No. of days after feeding	Autopsy findings	Remarks
1	25	13	25	Negative for *G. spinigerum*	Died
2	25	14	22	" " "	"
3	22	14, 15, 16	18	" " "	"
4	13	18, 20, 21	30	" " "	"
5	18	19, 20, 21, 22	1	" " "	"
6	21	20, 21	31	" " "	"
7	28	20, 21	10	" " "	"
8	7	21	5	" " "	"
9	18	28, 29, 30	32	" " "	"
10	20	28, 30	63	" " "	"

COMMENT

Group I, (table I) 8 cats (Nos. 1 to 8) were fed with infected cyclops containing a varying number of *G. spinigerum* larvae 7 to 10 days of age, and 3 (Nos. 9 and 10) were used as controls. All these animals were kept together in the same environment and their food and water were carefully controlled. Experimental cats Nos. 3, 5 and 6 died 48, 35, and 71 days respectively after feeding and on autopsy 2 living immature gnathostomes were found in cat No. 6; one was

TABLE 3.—*Result of Feeding Experiment on Group III*

Cat No.	No. of *G. spinigerum* larvae in cyclops	Age in days of *G. spinigerum* larvae in cyclops	No. of days after feeding	Autopsy findings	Remarks
1	51	14	15	Negative for *G. spinigerum*	Died
2	20	14	150	" " "	Killed
3	41	14	58	" " "	Died
4	46	14	103	" " "	"
5	32	21	85	" " "	"
6	46	21	82	" " "	"
7	39	28	16	" " "	"
8	50	28	56	" " "	"

imbedded in the gastric wall at the cardiac region and one was free in the peritoneal cavity behind the stomach. Cats Nos. 3 and 5 were negative for gnathostomes. Cats Nos. 1, 2, 7 and 8 were killed 30, 97, 30 and 91 days respectively after feeding and only cat No. 8 showed one living immature gnathostome imbedded in the right side of the diaphragm, while the rest were negative. The control cat No. 9 died of hookworm infestation 23 days after the experiment and cat No. 10 was sacrificed at 101 days; neither on post-mortem examination revealed gnathostomes.

Group II, ten cats were fed with different numbers of *G. spinigerum* larvae aged from 13 to 30 days in cyclops and died from 5 to 63 days after feeding and none of them on autopsy was found to harbour the parasite.

Group III, 8 cats were fed with different numbers of *G. spinigerum* larvae from 14 to 28 days old in cyclops and from 15 to 150 days after feeding were found on autopsy negative for gnathostome infestation.

Only two out of eight experimental cats of group I were positive and this result may be attributed either to the feeding experiment or to previous infection. It seems probable that these animals were previously infected since they were not laboratory bred and had been kept only for a short period in the laboratory before use. Also the percentage of infection and the number of worms recorded was small. In addition our experience has shown that cats in Siam have a high percentage of natural gnathostome infestation and in a few instances cats proven negative by fecal examination have been found to harbour male adult parasites in the peritoneal cavity and in stomach nodules. At any rate the results of Group II and Group III were conclusively negative and these seem to prove beyond doubt that *G. spinigerum* larvae in the body cavity of the cyclop even as old as 30 days are not able to grow in the definite host into the adult stage. Evidently they require some animal to act as a second intermediate host and this problem was elucidated by the following experiment.

FEEDING EXPERIMENT ON FISH

Fresh water fish were first considered to be the possible second intermediate host of the parasite because they are closely associated with cyclops, and are the common food of the definite host. They are also consumed by natives occasionally in a half-cooked condition. One species of cat fish, *Clarias batrachus* (Linnaeus), was selected for the experiment and 19 were bought from a market near Siriraj hospital. Five of them were sacrificed as controls and on autopsy revealed no evidence of gnathostome infestation and 14 were used in the feeding experiment. Each of the experimental fish was placed in a wide-mouth jar containing a sufficient amount of fresh water and allowed to feed on various numbers of cyclops harbouring different numbers of *G. spinigerum* larvae from 1 to 21 days old. They were then killed and examined at various intervals after feeding. The results are shown in table IV.

TABLE 4.—*Result of Feeding Experiment on Clarias batrachus (Linnaeus) with Gnathostoma spinigerum larvae*

Fish No.	No. *G. spinigerum* larvae fed	Age in days of *G. spinigerum* larvae in cyclops	Period of feeding experiment	Autopsy findings of larvae of *G. spinigerum*	Remarks
1	34	7	2 days	Many living larvae without further changes in morphology found in the stomach muscle.	Killed
2	31	7	2 days	3 living larvae without further changes in morphology found in the stomach muscle.	Killed
3	47	7	2 days	Negative	Killed
4	62	9	6 days	2 living larvae without further changes in morphology found in the intestinal wall.	Killed
5	78	10	6 days	1 living larvae without further changes in morphology found in the stomach wall.	Killed
6	59	10	6 days	" ditto "	Killed
7	64	11	6 days	" ditto "	Killed
8	61	11	7 days	2 encysted grown larvae found in the abdominal wall. 2 encysted and 1 non-encysted grown larvae found in the body muscle. 12 living non-encysted larvae found in the stomach wall, one of which showed an increase in size and a strong large intestine, the rest had thin atrophic intestines and show no further change. 3 living non-encysted larvae found in the intestinal wall; all had very thin yellow intestines and show no further change.	Killed

TABLE 4.—(*Continued*)

Fish No.	No. G. *spinigerum* larvae fed.	Age in days of *G. spinigerum* larvae in cyclops	Period of feeding experiment	Autopsy findings of larvae of *G. spinigerum*	Remarks
9	86	14	6 days	1 encysted grown larva found in the body muscle. 1 living non-encysted larva without further change found in the stomach wall. 1 dead non-encysted larva found in the intestinal wall.	Died
10	86	14	1 day	Many living non-encysted larvae without further change in morphology found in the stomach wall.	Died
11	96	20	9	1 non-encysted grown larva found in the body muscle. Many living non-encysted larvae without further change in morphology found in the stomach and intestinal walls, some of which showed a little increase in size.	Killed
12	60	20	21 days	1 encysted grown larva found in the body muscle.	Killed
13	52	21	13 days	1 non-encysted grown larva found in the body muscle. 4 non-encysted grown larvae found in the stomach wall.	Killed Killed
14	62	21	16	1 encysted grown larva found in the mesentery. 1 non-encysted grown larva found in the body muscle. 3 living larvae without further change in morphology found in the gastric wall and three similar larvae found in the intestinal wall.	Killed

COMMENT

With the exception of fish No. 3 all the experimental fishes were positive for *G. spinigerum* larvae of various stages in different locations, but 6 fishes showed encysted and non-encysted grown *G. spinigerum* larvae mostly in the body muscle and only a few in the gastric wall and mesentery (table IV). The cyst (fig. 1) is flat, more or less spherical in shape, slightly yellowish-gray in colour and measures 1.14 mm. in diameter. The cyst wall is thin, semi-translucent and through it one can see a coil of parasite. The encysted form after dissection from the cyst wall shows active movement and measures 2.70 mm. in length and 0.29 mm. in breadth. It is pinkish in colour and provided with a cephalic bulb that measures 156.2 μ in width and 106 μ in length with 4 transverse rows of well-developed single-pointed spines and a pair of lips. The cuticle is transversely striated throughout and covered with small single-pointed spines which are numerous on the anterior half of the body and become gradually less in number and disappear entirely at about the posterior third. The alimentary system consists of a strong muscular esophagus, a well-developed intestine lined with cells containing coarse yellowish granules, and a subterminal anus. A

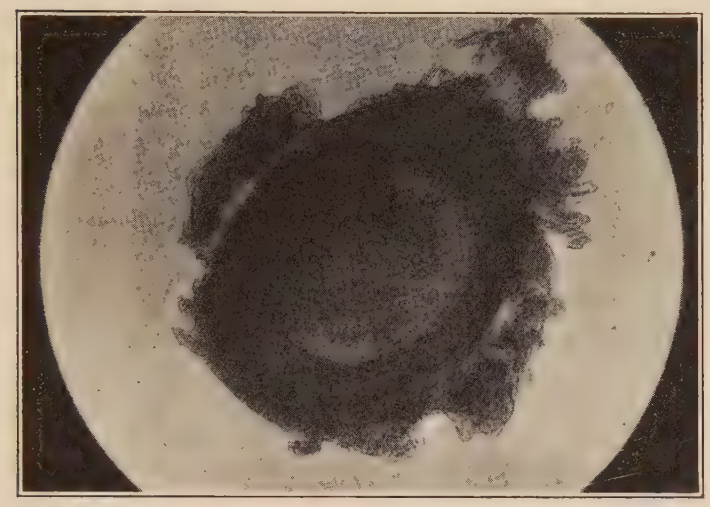

FIG. 1. Microphotograph of a cyst of *G. spinigerum* larva found the body muscle of *Clarias batrachus* (Linnaeus).

long narrow colourless column of small, finely granular cubical cells is seen in the body cavity which is probably the rudimentary germinal organ. Two pairs of transparent colorless cervical sacs are present in the anterior portion of the worm (fig. 2).

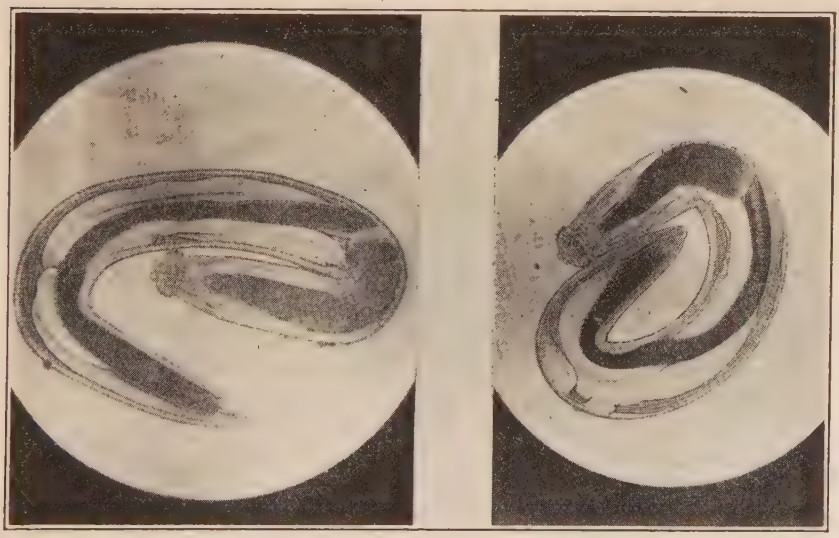

FIG. 2. Microphotograph of a *G. spinigerum* larva dissected out from a cyst.
FIG. 3. Microphotograph of a non-encysted grown *G. spinigerum* larva found in the body muscle of *Clarias batrachus* (Linnaeus).

The non-encysted grown larvae (fig. 3) measure from 1.10 mm. to 1.28 mm. in length and 0.11 to 0.16 mm. in breadth and have the general appearance and structure of the encysted form.

The non-encysted and not yet developed larvae found in the stomach and intestinal walls had size and morphology identical with those of the larvae in the body cavity of the cyclops that were fed, but some of them show atrophic slender intestines. Evidently these larvae had been ingested by fishes only for a short period before the examination was made.

This experiment shows clearly that the *G. spinigerum* larvae in cyclops when ingested by *Clarias batrachus* are able to penetrate the gastrointestinal wall of the fish and finally develop in the body muscle into an encysted form. But the question may be raised as to why only a small number of *G. spinigerum* larvae in cyclops could develop to non-encysted and encysted grown larvae in 6 experimental fishes. It may be explained that those negative fishes were allowed to feed on young larvae and on the other hand they were killed within a short time after the experiment. The larvae may not have yet reached the infective stage or probably had no time to undergo further development. Even though they have attained similar morphology, probably not all of them possess equal vitality. It was found that not all infected cyclops fed were taken by the experimental fishes at the same period, since various stages of *G. spinigerum* larvae have been found in an individual fish and on a few occasions some dead and some living infected cyclops were recovered from the water of the experimental jars.

SUMMARY

1. Attempts made to infect cats with *G. spinigerum* larvae aged 7 to 30 days in cyclops by feeding gave negative results.

2. The feeding experiments on cats show clearly that *G. spinigerum* requires a second intermediary host in its life-cycle.

3. *Clarias batrachus* (Linnaeus) (fresh water fish) is definitely found to be one of the second intermediate hosts of *G. spinigerum*, because in the muscle of this fish *G. spinigerum* larvae that have developed in cyclops are able to develop into an encysted form.

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A REPORT ON EXPERIMENTAL INFECTIONS OF *OVIS ARIES* WITH THE INFECTIVE LARVAE OF *OSTERTAGIA CIRCUMCINCTA**

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I. INTRODUCTION

The experiments described in this paper were planned to determine the pathogenicity of the infective larvae of *Ostertagia circumcincta* on the host, with particular reference to the blood. Not only was this phase of the work deemed important as a means of determining the rôle of this parasite in the raising of sheep, but also it was thought that some interesting observations might result in view of the fact that very little work of this nature has been recorded concerning the many species of strongyloid forms which are known to be present in the alimentary tract of ruminants.

The results reported in this paper, while limited in some respects, are submitted with the idea that further work based on the experience obtained from these studies may be undertaken.

It should be noted throughout this procedure that the establishment of infection with pure strains of parasites in sheep maintained under laboratory conditions does not approach the natural conditions to which an animal is subjected on pastures. Furthermore, infections by one specific type of parasite rarely, if ever, occur in nature, and it is mixed infections which probably create the most havoc in the host.

II. REVIEW OF LITERATURE

Some observations have been published which indicate that parasites related to *O. circumcincta* affect the blood of the host and also that factors may be present under certain conditions which may alter the normality of the blood of man and other species of animal life.

Fourie (4) in 1931 published a report showing the effect of *Haemonchus contortus* on the blood of sheep. His work indicated that in some instances large doses of this nematode were responsible for the death of the experimental animals, in which the outstanding symptom was extreme anemia. Pathologic changes in the blood were evident in a number of animals which did not die.

Sarles (7) observed that young dogs given large doses of hookworm larvae, either cutaneously or orally, died shortly afterward of severe anemia. He showed that old animals were much more resistant than

* Departmental contribution No. 29.

young animals toward hookworm infections. In experiments with older dogs, the time required for the parasites to reach the adult stage was longer, and fewer eggs were deposited. Sarles's conclusions indicated that following cutaneous or oral infection in old dogs there was an immediate increase in the total number of white cells. This was accompanied by a decrease of eosinophiles followed by a marked eosinophilia. There was an attendant decrease of lymphocytes and mononuclears. The red corpuscles in young dogs showed a sharp decrease in number about the ninth day after infection (dog number 413) and the percentage of hemoglobin was correspondingly lower. Young dogs receiving large doses of hookworms died from a rapidly progressing anemia before a change occurred in the total or differential white cell counts.

Schwartz (9) confirmed some results of previous workers and concluded that certain of the nematode parasites secrete hematoxins. The parasites with which he worked were *Ascaris lumbricoides, Ancylostoma caninum, Bunostomum phlebotomum, Haemonchus contortus, Trichuris depressiuscula,* and the cestode, *Thysanosoma actinoides.*

Cort (3) and his coworkers showed that while a reduction in hemoglobin may be connected with hookworm infection in men, this did not necessarily show the extent of the infection. It was concluded that there were many probable factors which caused variation; namely, race immunity, acquired toleration, food, and conditions of living.

Neser (6) has shown that the content of blood varies in normal animals. In his work on the blood of equines he found that regular exercise greatly increased the number of erythrocytes in these animals. Some race horses in full training had three times as high a red-cell count as stabled horses. A normal stabled horse, upon receiving regular exercise showed an enormous increase in the red-cell count in five weeks. He found that the reverse change with idleness was slow.

Canham (2) found, as did Neser in horses, that the red-cell count of cattle was increased with exercise. Canham showed also that grade cattle had higher red-cell counts than pure bred cattle under the same conditions. He pointed out that pregnancy, oestrum, and high altitude tend to increase the number of erythrocytes in cattle.

Stoll (10) from his studies of *Haemonchus contortus* summarizes as follows: "An experiment is reported in which two helminth-free lambs, upon fenced-in pasturage permitting natural repeated infection, during the summer developed an initial dose of *H. contortus* larvae, first as accumulation of parasites, and then a self-cure which expelled the worms and protected the animals thereafter against any significant amount of further infestation with this stomach worm. The rise and fall of the infestations were followed by enumerating the eggs in the feces, and the basis of this procedure is set forth. The parasitic test of this acquired

resistance is given and the bearing of the concept emphasized in relation to host-parasite relationship with helminths."

III. EXPERIMENTAL WORK

A. Methods and Technique

The blood samples of sheep used for examination in the experiments herein described were obtained from the veins of the ear of the animals. The ear was shaved and washed, a vein punctured by a needle, and the sample taken from the freely flowing blood. The total cell counts were made by the use of an American Standard Haemacytometer. The hemoglobin was estimated by a Dare hemoglobinometer until December, 1932, when it was replaced by a Newcomer hemoglobinometer.

The differential counts were made from blood smears stained with Wright's stain. The percentages found were based upon results of counting from 150–300 cells.

In order to insure pure cultures of *O. circumcincta* larvae, it was first necessary to establish this infection in sheep free of other gastro-intestinal parasites. This was accomplished by securing lambs within 48 hours after birth, and raising them on pasteurized milk from sterile bottles. Upon weaning, the lambs were given hay and grain that had been previously sterilized in an autoclave from three to six hours at 15 pounds pressure. The animals were kept in clean pens with raised floors, and the pens were treated frequently with live steam and three per cent cresol solution. The animals received approximately the same amount of milk and grain but were allowed alfalfa *ad libitum.*

The method described by Threlkeld (15) for obtaining the larvae for the initial infection of the lambs was followed and systematic fecal examinations were made on the experimental animals for several weeks before they received the initial dose of infective larvae. None of these animals showed previous infection.

Three methods of fecal examinations were used. In the first method the fecal material was broken into small pieces in water and freed of fiber by pouring through a small meshed strainer. This material was transferred to a test tube which was filled nearly full with 30 per cent sodium chloride solution. The resulting mixture was then centrifuged at 2000 r.p.m. for one minute. By the use of a blunt glass rod, the film from the top of the centrifuged solution was transferred to a glass slide, covered with a cover slip and examined systematically under a microscope. This method of fecal examination was employed to ascertain whether the lambs were showing a positive egg count before infection experiments were initiated. After the first dose of larvae had been administered the Stoll (13) D. C. F. procedure was used until the egg count

reached 1000 eggs per gram of feces. At this point the D. C. F. and dilution method Stoll (13), were employed simultaneously. The counts of 5000 eggs 7/4/33 and 6000 eggs 6/29/33 (Table 1) represent the readings obtained by the dilution method. In every case only the highest readings were recorded.

The method employed for the determination of the number of larvae appearing in the cultures, and the manner in which these larvae were administered to the experimental animals were identical in all cases with the procedure described by Threlkeld (15).

B. Results of Infections

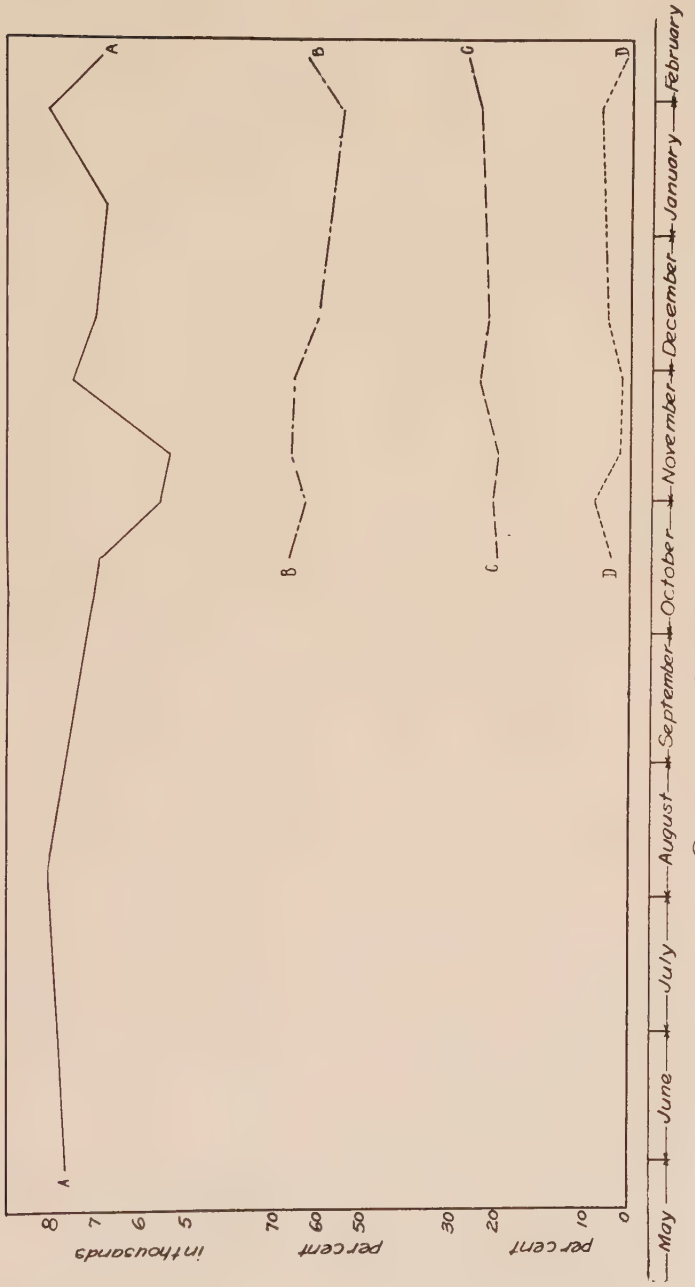
An effort to produce infections with *O. circumcincta* larvae in eight lambs was made in which it was expected that severe and unmistakable symptoms would result. This was not accomplished, however, and pathologic developments were noticeable only to a certain degree. In one instance only was there secured an infection seemingly severe enough to cause very apparent symptoms of unthriftiness.

The complete records of all the animals were placed on graphs but only those for three are included, *viz.*, sheep 2 (graph 2 and 2a), sheep 6 (graph 6 and 6a) and sheep 8 (graph 8 and 8a). Table I contains the most essential data relative to the entire group of animals experimentally infected.

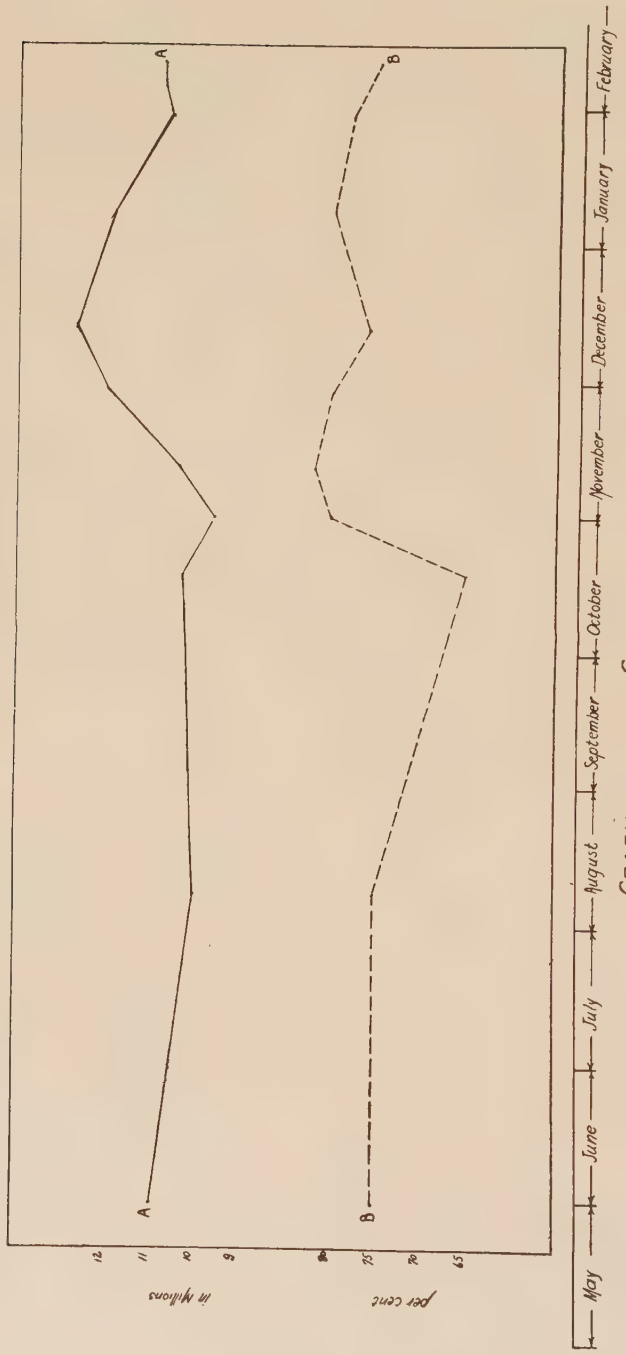
DISCUSSION

Since the ultimate aim of the experiments outlined in this study was to secure definite evidence of parasitic infection, in the host animals four thousand, three hundred and ninety-five infective larvae were administered orally to the first lamb as a test dose.

As no pathologic results were obtained from this administration, the doses of larvae thereafter fed were increased and the time intervals in the various cases purposely varied. The data recorded in column 3 of Tabl I, as far as the proportionate number of larvae given is concerned, bear little significance to the problem other than they indicate the numbers of larvae required to cause pathologic symptoms in lambs 6 and 8. The information, however, afforded by the entire table might suggest a few interesting points for discussion. First, with the exception of lamb 7, oral administrations of infective larvae extending over a prolonged duration to lambs 1, 4 and 6 produced egg counts consisting of a gradual increase to a maximum and then a gradual decrease to a minimum. These counts extended for periods of from approximately four to six months. On the other hand, two doses of larvae administered to lambs 5 and 8 respectively, over a period of one week produced a comparatively high egg count but after the maximum egg count was attained there was a rapid drop in the numbers of eggs to zero with no positive counts



GRAPH 2, Control, showing the courses of white cells and differential counts.



GRAPH 2A, Sheep 2, Control, showing the courses of the red cells and hemoglobin.

appearing thereafter. These unusual counts in lambs 5 and 8 might indicate the establishment of immunity. An indication of immunity is further manifested by a gradual rise of eosinophiles in the blood of lamb 5, which ultimately reached seven per cent on the day after the first positive egg count. The significance of this is further emphasized since a low normal of two to two and one-half per cent had been established for several weeks in this lamb before it had received doses of infective larvae. In lamb 8 (Graph 8) there is a marked increase in the number of eosinophiles one week after the final positive egg count on July 18, 1933. No other lamb which had been infected exhibited this rise in eosinophile count which is generally considered as a factor in the development of immunity. None of the experimental animals were subjected to further infection, hence no positive conclusions can be drawn.

Certain data relative to the examinations of blood procured from normal sheep as reported by Burnett (1) can hardly be taken as representing the conditions under which our control animal 2 was maintained. Apparently no consideration was given by the investigators as to whether the samples were taken from parasite-free animals or whether they were taken from range animals. Hence, the interpretation of the degree of the severity of the infections which were established in the experimental animals must be based upon a comparison with the blood picture as represented by the control lamb 2.

Sheep 8 received 225,000 larvae between May 29, 1933, and June 7, 1933. Compared to the number of larvae given to previous animals, with the exception of sheep 6, this was an enormous dose and was administered in a short period of time. A study of the hematologic records, graphs 8 and 8A show significant departures from the normal as established by the control animal 2 and the entire group of animals before infective larvae were administered.

From June 20 to July 11 this animal gained but one pound, whereas before it was infected and after the infection had decreased in severity, as on July 18, the weekly gain was from three to four pounds. This interruption in growth seems to bear a close connection with the fact that on June 20 the fecal examinations became positive and reached a peak of 5,000 eggs per gram during the week of July 4. Graph 8A shows a red-cell change of no significance other than that it dropped slightly. Between the dates of June 14, 1933, and July 25, 1933, there was a slight drop in the percentage of hemoglobin. The white-cell count indicated no consistent abnormal change during this time, but on July 4 it showed a count of 12,880 per cubic millimeter, which was 5,863 higher than the average of control animal 2. The differential count is of some interest in that the lymphocyte percentages decreased steadily from 63.3 per cent on June 27 to 39.3 per cent on July 25. The neutrophiles showed no in-

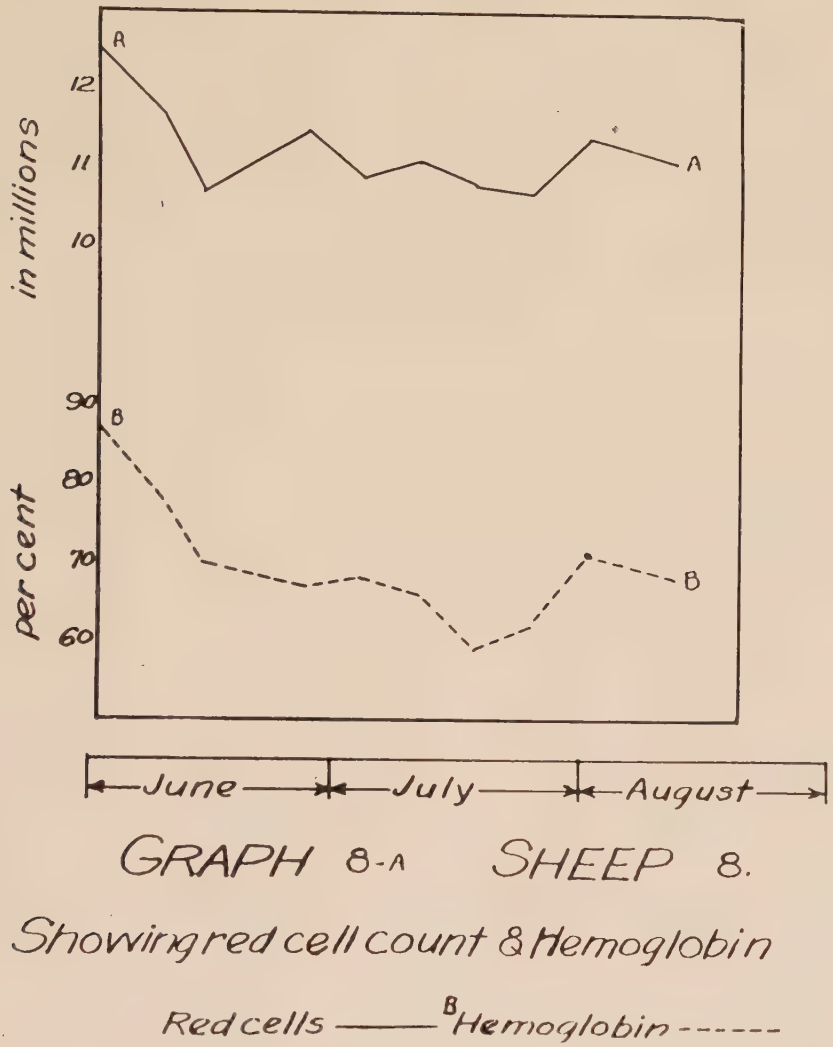
GRAPH 8. SHEEP 8.

Showing white cells & differential count

A White cells ——— C Neutrophiles— — —
B Lymphocytes — — — D Eosinophiles --------

GRAPH 8, Sheep 8, showing the courses of the white cells and differential counts. This animal received doses of infective larvae as indicated below.

5/30/33 – 140,000 – 6/6/33 – 85,000
Total 225,000



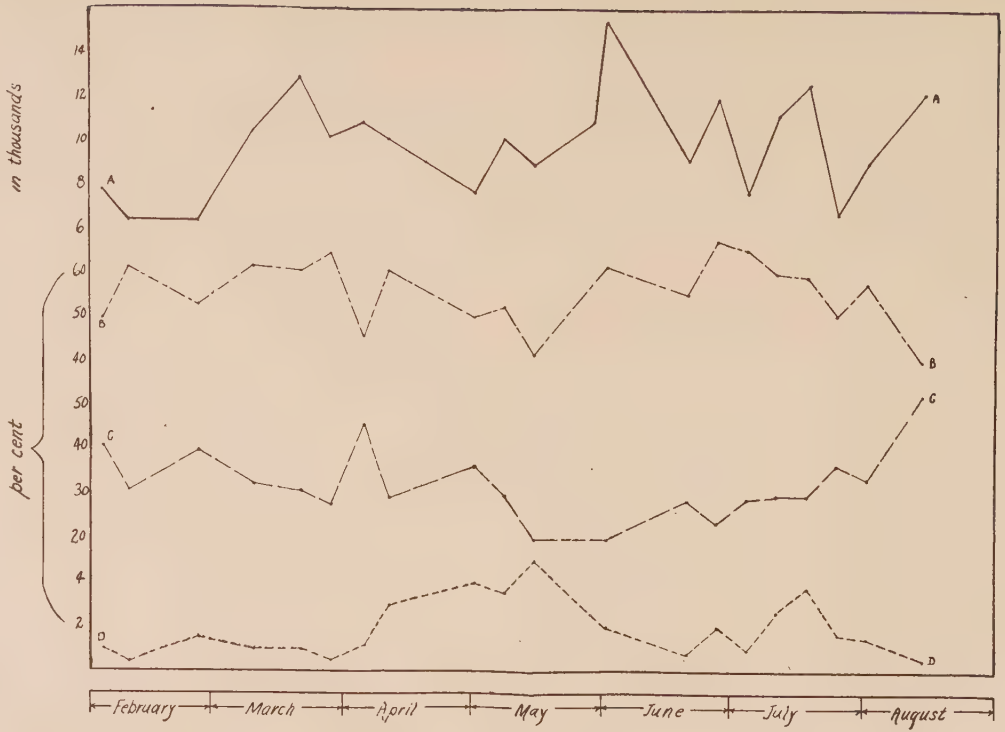
GRAPH 8A, Sheep 8, showing the coures of the red cells and the hemoglobin. Doses of infective larvae as recorded in Graph 8.

crease. From July 4 to August 1, a few hemocytoblasts or undifferentiated cells, probably precursors of erythrocytes, appeared. The eosinophiles showed a rise from a low percentage to 20.8 on July 25, and then dropped below the average as maintained by the control animal 2. This lamb showed some visible indications of parasitization. Its coat became dry and rough. The abdomen was distended and the animal suffered intermittently from constipation. These symptoms, however, were only transient.

TABLE 1.—*Degree of infection, weight loss and egg output of lambs experimentally infected with O. circumcincta larvae*

No.	Age when infected	No. of larvae given and date of infection	Greatest weight loss and time of occurrence	Duration of egg count. Greatest egg count per gram of feces and date	Blood changes
1	4 months	6/19/32 – 170 : 6/26/32 – 1,100 7/ 3/32 – 200 : 7/10/32 – 400 7/17/32 – 100 : 7/24/32 – 700 7/31/32 – 475 : 8/ 7/32 – 700 8/21/32 – 100 : 8/28/32 – 450 Total larvae fed – 4,395	* 4 lbs. 10/30/32 to 11/6/32	150, 12/4/32 7/11/32 to 12/18/32 5 months, 7 days	None
2	Control		* 3 lbs. 10/24/32 to 11/6/32		None
3	6 months	9/16/32 – 1,000 : 9/23/32 – 9,000 9/30/32 – 13,000 Total larvae fed – 23,000	* 4 lbs. 11/4/32 to 11/11/32	350, 1/21/33 10/7/32 to 2/3/33 3 months, 26 days	None
4	5 months	8/12/32 – 1,600 : 8/26/32 – 585 9/23/32 – 600 : 10/ 4/32 – 4,000 10/21/32 – 3,000 : 11/ 5/32 – 2,500 11/12/32 – 2,000 : 11/19/32 – 2,400 11/26/32 – 6,500 : 12/ 3/32 – 20,000 12/10/32 – 3,500 Total larvae fed – 46,685	* 6.5 lbs. 11/5/32 to 11/19/32	40, 9/23/32 9/9/32 to 12/30/32 3 months, 21 days	None
5	7 months	10/ 7/32 – 28,000 : 10/14/32 – 27,000 Total larvae fed – 55,000	* 4.5 lbs. 11/4/32 to 11/18/32	400, 12/16/32 10/28/32 to 12/16/32 1 month, 28 days	Slight
6	3 weeks	2/ 9/33 – 25,000 : 2/16/33 – 1,000 3/16/33 – 160,000 : 3/23/33 – 160,000 3/30/33 – 160,000 : 4/ 6/33 – 160,000 4/13/33 – 160,000 : 4/20/33 – 160,000 4/27/33 – 160,000 : Total – 1,146,000	6.5 lbs. 4/20/33 to 5/11/33	6,000, 6/29/33 1/23/33 to 7/27/33 6 months, 4 days	Yes
7	1 month	2/16/33 – 8,100 : 2/23/33 – 1,000 3/ 2/33 – 4,100 : 3/16/33 – 14,500 Total larvae fed – 27,700	No loss	100, 3/28/33 3/9/33 to 4/30/33 1 month, 21 days	None
8	1 month	5/30/33 – 140,000 : 6/ 6/33 – 85,000 Total larvae fed – 225,000	Gained 1 lb. as against 3 and 4 lbs. before and after these dates 6/20/33 – 7/11/33	5,000, 7/4/33 6/20/33 to 7/18/33 28 days	Yes

* Poor grade of hay fed at this time probably responsible for some loss of weight.



GRAPH 6, Sheep 6, showing the courses of the white cells and differential counts. This animal received doses of infective larvae as indicated below.

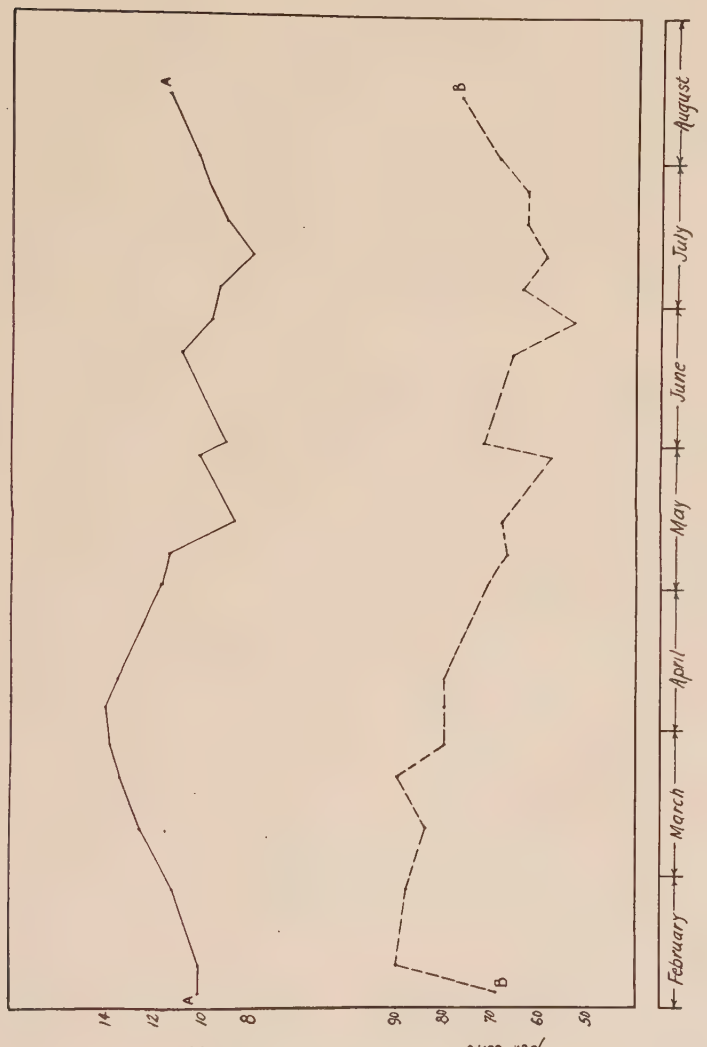
2/ 9/33 – 25,000 – 2/16/33 – 1,000
3/16/33 – 160,000 – 3/23/33 – 160,000
3/30/33 – 160,000 – 4/ 6/33 – 160,000
4/13/33 – 160,000 – 4/20/33 – 160,000
4/27/33 – 160,000 – Total . 1,146,000

Sheep 6 received 1,120,000 larvae between March 16 and May 4, 1933. It had received previously, between Fbruary 8 and 16, 1933, 26,000 larvae. Although the results obtained were by no means marked, some interesting symptoms were noted. Graphs 6 and 6A give the hematologic records, and Table I the degree of infection, dates larvae were administered, loss of weight and greatest egg burden and age records. A study of the graph 6A shows that there was an increase in the erythrocyte count, which Fourie had noted to occur in the haemonchosis of sheep. There was no particular evidence of this rise in the number of red cells n lamb 8, but it did occur in some of the animals which had received lighter administrations of larvae and whose records are not here recorded. There was a decided drop in the red-cell count and the hemoglobin percentage between April 11 and August 1. The average red-cell count between these dates was 9.9 millions per cubic millimeter, while the average count for the entire period in which the animal was under observation was 10.98 millions, a difference of 1.08 millions per cubic millimeter. The average for the control animal was 10.92. The average hemoglobin percentage between April 11 and August 1 was 64.0, while before and after this time, it was 80.7 per cent, a difference of 16.7 per cent. The final dose of the 1,120,000 larvae was administered on April 27.

The white-cell counts of this animal (sheep 6), while unusually high, were very erratic. On June 1, the white-cell count was 15,450. The differential count offers some points of interest. After April 11, the lymphocytes dropped gradually from 61 per cent to 42 per cent on May 15, after which time there was a gradual increase until August 14. The neutrophile percentage showed much variation, but little significance. The eosinophile percentage was slightly high from May 1 to May 15. A record of the monocytes indicated very little variation. From May 1 to June 1 hemocytoblasts appeared.

The interruption of growth of sheep 6 showed as in sheep 8, evidence of pathologic disturbance, is correlated with the egg burden and this connects the disturbance with the parasitic infection. From February 23 to March 9 there was a comparatively slight egg output and after this time the fecal examinations remained negative for three weeks. From April 20 to May 11 (see Table I) the weight indicated a loss of six and one-half pounds. During this time the egg output of sheep 6 reached 800 per gram of feces and on June 29 it reached a maximum of 6000 eggs per gram of feces, or approximately 2,500,000 eggs per day.

The changes noted above were definitely pathologic. This conclusion is further supported by the facts that the sheep had a dry, rough coat; was pot-bellied, and cachetic. It was subject to intermittent spells of con-



Graph 6A, Sheep 6, showing the courses of the red cells and the hemoglobin. Doses of infective larvae as recorded in Graph 6.

stipation, and during these periods a shallow, rapid respiration was apparent.

In sheep 8 and sheep 6, undifferentiated cells appeared, probably hemocytoblasts. The initial onset of anemia which is manifested simultaneously by a drop in the red-cell count and a drop in the percentage of hemoglobin, and by crenation of the red-cells, as well as the presence of many intermediate stages of immature erythrocytes, indicates that these undifferentiated cells are red-cell precursors.

It is possible that some of these undifferentiated cells may have been included in the white-cell count which would explain their rapid increase as indicated by the graphs. The simultaneous drop in the lymphocytes and increase in eosinophylles together with the fact that the neutrophylles are unaffected present a typical picture of a parasitized animal.

CONCLUSIONS

1. Light infections with *O. circumcincta* larvae (4395–27,700) given over periods of two weeks to two months and nine days, to lambs 1, 3 and 7, four months, six months and one month old, apparently caused no injury to the host.

2. Moderate numbers of *O. circumcincta* larvae (46,685) given over a period of four months to lamb 4, five months old, other than a loss in weight, caused no pathologic symptoms.

3. Moderate numbers of *O. circumcincta* larvae (55,000) given over a short period of time (one week) to lamb 5, seven months old, produced a slight eosinophilia of a short duration.

4. Large numbers of *O. circumcincta* larvae (225,000) given to lamb 8, one month old, over a period of one week, affected the growth of the host animal. There was a decrease in the percentage of hemoglobin. The differential blood count indicated a decrease in the percentage of lymphocytes, a pronounced eosinophilia and the appearance of undifferentiated cells designated as hemocytoblasts; the latter probably are called forth as red-cell precursors to counteract the anemia.

5. Very great numbers of *O. circumcincta* larvae (1,145,000) given to lamb 6, three weeks old, over a period of two months and eighteen days caused a loss of weight and produced pathologic changes with erythrocytosis, followed by a decrease in the red cells, as well as a drop in the hemoglobin percentage and the number of lymphocytes. Hemocytoblasts appeared at this time.

6. Moderate doses of larvae (55,000) and heavy doses of larvae (225,000) given over a period of one week to lambs 5 and 8, seven months and one month old respectively, yielded a comparatively heavy egg output over short duration, twenty-eight days to one month and twenty-eight days which rapidly reached a maximum and rapidly de-

creased to permanent negative counts. This was a marked contrast to the histories of egg count readings obtained from lambs 1, 4, and 6 which received doses of infective larvae over extended periods.

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THE RECOGNITION OF A NEW ORDER IN THE ACANTHOCEPHALA*

Harley J. Van Cleave

The period dating from 1892, when Hamann proposed the recognition of three families among the Acanthocephala, marked the beginning of a new era in acanthocephalan studies.[1] Since that date a voluminous literature has made its appearance. Out of the constantly growing catalog of species and genera various writers have attempted to create a taxonomic system which would reflect phylogenetic relationships. The results have varied widely. Several authors have been unwilling to make broad generalizations and have in consequence confined their attempts to the recognition of family relationships, emending and expanding the basic classification of families laid down by Hamann. Others have frankly wrestled with the problem and have attempted to create a perfect system including all taxonomic categories even when fundamental morphological details were in many instances lacking and the known facts were too often misinterpreted.

Lauro Travassos, in a long series of systematic papers on the Acanthocephala culminating in a survey of the entire group in 1926, resognized a system which made no attempt at creation of natural groups higher than the families. His four families perpetuated the three concepts of Hamann to which he added a fourth family, the Rhadinorhynchidae. Travassos's allocation of genera to his family categories indicated a keen appreciation of significant characters. Errors of the present writer and of several other workers were set right by him as, for example, his recognition of the genus *Mediorhynchus* as agreeing with the characters enumerated for the Gigantorhynchidae. While not all of his decisions

* Contributions from the Zoological Laboratory of the University of Illinois, No. 478.

[1] In a footnote, Meyer (1932: 21) has expressed the opinion that the process of family construction is synthetic and therefore Hamann's pioneer attempts at family recognition have but historical value since his results grew out of an analytical consideration of genera. The present writer cannot share this belief. Every category in the taxonomic system, from subspecies onward, necessitates both analytical and synthetic treatment. The dual goal of all taxonomy on the one side looks toward the bringing together of those forms which are alike, regardless of whether they are individuals or families, and on the other side seeks to separate those forms which have significant differences irrespective of the taxonomic level at which the study is being pursued. In any attempt at deriving a natural system neither the analytical nor the synthetic aspect may be ignored. The synthetic process in group formation is all too frequently the sole tool of the novice who finds occupation in the attempt at collating the researches of earlier systematists without possessing the essential analytical background of first-hand experience and morphological training to give depth and value to his synthetic product.

as to synonymy can be accepted as valid, he displayed a fundamental knowledge of morphology which gave weight to his decisions.

Among the highly varied results of attempts at fabricating a unified taxonomic system for the Acanthocephala little can be done toward harmonizing the various systems. One of the earliest contributions of significance was the paper by Southwell and Macfie (1925) setting forth a result which gives many evidences of artificiality. In fact their contribution consists largely in the elevation of Hamann's original three family concepts to subordinal rank and in attempting to subsume all subsequently recognized genera under these three categories.

Faust (1928) made no direct contribution to the analysis or synthesis of the group in his "Human Helminthology" but felt impelled to create two new names, Gigantorhynchata and Echinorhynchata, for concepts already in very general use. One reason for creating these terms seems possibly to have been the desire to advocate terminations for ordinal names uniform with those adopted in his section on digenetic trematodes.

Rauther (1929) in compiling the section on Acanthocephala in Kükenthal's "Handbuch der Zoologie" adopted a classification based on the work of Travassos. He superimposed a single ordinal name, "Echinorhynchoidea," of exactly the same inclusion as the class Acanthocephala. This introduction of a new name gave a superficial aspect of completeness without making any real contribution to the causes of taxonomy and nomenclature.

In 1931, A. Meyer formulated a taxonomic system which he perfected and extended in his treatment of the Acanthocephala in Bronn's "Tierreichs" (1932 and 1933). Basically, this system sought to establish new sets of morphological and biological characters in drawing diagnoses for groups above the status of family. Meyer was the first to effectually utilize characteristics provided by: (1) the lacunar system of the subcuticula; (2) the arrangement of the proboscis hooks; (3) the protonephridial organs; (4) the ligament sacs within the bodies of the females; and (5) host-habitat relationships in the developmental cycle, in diagnosing groups of ordinal rank. He established two orders, the Palaeacanthocephala and the Archiacanthocephala, which represent by far the most sound system advocated by any investigator.

However, in synthesizing his system Meyer has focused his attention so sharply on the unifying nature of certain characteristics that he has failed to appreciate the distinctive value of other morphological features to the end that some families and genera are included in his system only at the cost of marked exception to statements itemized in his enumeration of characteristics. Thus, for example, histological details of the cement glands of the males and specific plan of construction of the walls of the

proboscis receptacle in some genera and families provide characters for grouping and for separating ignored by Meyer.

As an instance of inconsistency resulting from too close adherence to one set of characters and ignoring others, may be cited his inclusion of the family Neoechinorhynchidae in his order Archiacanthocephala. By so doing he has thrust a group bound to the aquatic habitat into an assemblage otherwise distinguished as occurring in terrestrial hosts. This biological incompatibility is directly paralleled by fundamental morphological differences which mark the Neoechinorhynchidae as distinct from the ordinal concept Archiacanthocephala.

A group of genera which the present writer has always considered as having fundamental resemblances consists of Hebesoma, Pallisentis, Farzandia, Acanthosentis, Neosentis and Quadrigyrus. Despite the varying degrees of difference expressed in generic diagnosis and family description, all of the foregoing have points in common, most of which they share with the Neoechinorhynchidae. Meyer has disregarded these common points or has considered them as of less significance than others and in consequence places most of the foregoing genera in his Palaeacanthocephala, relegating Hebesoma and the Neoechinorhynchidae to the Archiacanthocephala.

On the basis of inconsistencies of this nature the writer has concluded that the two ordinal categories of Meyer are insufficient to reveal the actual phylogenetic relationships of all acanthocephalan genera and families, for a third ordinal group must be recognized to accommodate these related forms mentioned above, none of which can be admitted to either of Meyer's orders. It is the object of this paper to propose and diagnose a third order for the Acanthocephala to which the name Eoacanthocephala is here applied. In the recognition of this order most of the disharmonies of the Meyer ordinal concepts disappear and frequent reference to exceptions in his characterizations are eliminated.

In the following section the new order will be diagnosed and its inclusions will be set forth.

EOACANTHOCEPHALA, NEW ORDER

Longitudinal vessels of the lacunar system of the body wall essentially dorsal and ventral. Body covering with or without cuticular spines. Subcuticula containing a few ovoidal or amoeboidal giant nuclei. Ligament sacs of females usually distinct, one dorsal, one ventral. Specialized nephridial organs lacking. Cement gland of male a single syncytial mass containing giant nuclei. Proboscis hooks radially arranged, without pronounced asymmetry on various surfaces. Hook formula constant for most species. Wall of proboscis receptacle containing a single muscular layer. Embryos ellipsoidal, membranes not heavy. All

definitive hosts aquatic and with exception of one species (*Noeochinorhynchus emydis* in turtles) parasites of the digestive tract of fishes. Geographical distribution includes all the continents.

Two suborders, four families and twelve genera are included in the Eoacanthocephala as set forth in the following tabular review of the complete scheme for classification of the Acanthocephala in which Meyer's orders are emended by omission of forms transferred to the Eoacanthocephala.

A. Order Palaeacanthocephala Meyer, 1931 (emend.) as restricted by removal of Quadrigyridae, recognition of the genus Farzandia as a synonym of Pallisentis, and removal of part of Meyer's concept of the genus Rhadinorhynchus to the order Eocanthocephala.

B. Order Archiacanthocephala Meyer, 1931 (emend.) as restricted by removal of Meyer's concept of the family Neoechinorhynchidae.

C. Order Eoacanthocephala Van Cleave, 1936 (new).

a. Suborder Gyracanthocephala (new) Van Cleave, 1936. Eoacanthocephala with body spines.

I. Family Pallisentidae Van Cleave, 1928.

1. Genus Pallisentis Van Cleave, 1928 (syn. Farzandia Thapar, 1931).
2. Genus Acanthosentis Verma and Datta, 1930.
3. Genus Neosentis Van Cleave, 1928.

II. Family Quadrigyridae Van Cleave, 1920.

4. Genus Quadrigyrus Van Cleave, 1920.

b. Suborder Neoacanthocephala (new) Van Cleave, 1936. Eoacanthocephala without body spines.

III. Family Neoechinorhynchidae Van Cleave, 1919.[2]

5. Genus Neoechinorhynchus Hamann in Stiles and Hassall, 1904.
6. Genus Tanaorhamphus Ward, 1917.
7. Genus Gracilisentis Van Cleave, 1919.
8. Genus Octospinifer Van Cleave, 1919.
9. Genus Pandosentis Van Cleave, 1920.
10. Genus Eosentis Van Cleave, 1920.
11. Genus Atactorhynchus Chandler, 1935.

IV. Family Hebesomidae Van Cleave, 1928.

12. Genus Hebesoma Van Cleave, 1928.

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[2] First date for use of name. Generic concept dates from Hamann, 1892, and type of family was renamed by him in 1904 in Stiles and Hassall but family was not renamed at that time.

SUMMARY

Attempts at classifying the Acanthocephala in categories above the family are reviewed.

The classification of Meyer (1931) is emended by recognition of a third order, the Eoacanthocephala, which is characterized chiefly by the possession of syncytial cement glands in the male and of a single muscular wall in the proboscis receptacle.

Two suborders of this new order are recognized, the Gyracanthocephala having body spines and the Neoacanthocephala without body spines. The included genera and families are listed.

ENERGY AND FOOD RESERVES IN THE DEVELOPMENT OF NEMATODES*

ARNALDO GIOVANNOLA

(Roma, Italy)

In 1901 Weinland discovered that in the body of parasitic nematodes glycogen is present in a very great quantity. This author calculated that the glycogen present in the adult *Ascaris lumbricoides* is from 15 to 47 per cent of the dry residue of this worm. Weinland was able to determine that these worms use each day 0.7 g. of glycogen for each 100 g. of weight. Living ascarids in one per cent solution of NaCl he found consumed glycogen and produced CO_2, valeranic and other fatty acids. He also observed that these parasites live better in a medium without oxygen than in one provided with O_2 and came to the conclusion that the disappearance of glycogen is connected with a fermentation of this substance and not with an oxidation. Later investigations confirmed the findings of Weinland, and Brault and Loeper (1904) calculated that there was 0.968 g. of glycogen in a specimen of *Ascaris lumbricoides* with a total weight, in the fresh state, of 39.50 g.

Other authors studied another substance present in the body of parasitic nematodes, *i.e.*, fat. Recently Payne (1922 and 1923) has shown a relationship between the vertical migration of infective larvae of *Necator americanus* and the disappearance of granules of fat present inside these larvae. Goodey (1930) had occasion to see the revival in water of larvae of *Tylenchus dipsaci* which had been kept for six years in a dry condition and came to the conclusion that some reserve substance must have been of very great importance in this phenomenon. Goodey was able to show the fatty nature of these reserve substances.

So it seemed interesting to me to go over the most important stages of the life cycle of nematodes to study their reserves of food and the relation of these reserves to their activity. In this paper the results of these investigations are reported.

MATERIAL AND TECHNIQUE

The presence of glycogen was determined by employing the Best stain, Lugol iodine solution, and the Bauer staining test. This last, intro-

* From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University. This work was done under the auspices and with the help of the International Health Division of the Rockefeller Foundation. The writer wishes to express his thanks to Dr. W. W. Cort, under whose direction the work was carried out, and to Dr. G. F. Otto for their helpful suggestions. He is also indebted to Mr. K. B. Kerr and Dr. D. A. Porter for help in obtaining certain of the material for study.

duced by Bauer (1933), was successfully used in the study of glycogen in protozoa (Giovannola, 1934) and has been successfully employed in the present researches. The technique of the Bauer staining test is briefly as follows: After fixation in Carnoy the slides are kept for some time in a solution of celloidin in alcohol-ether to avoid the loss of glycogen in further passages. Then they are passed through alcohol at 60° C., kept for an hour in a 4 per cent solution of chromic acid in the dark room and then washed in water. They are then placed for 30 minutes in a solution of fuschin (Wermel reagent employed in Feulgen test), and transferred for a few minutes into a solution of sodium bisulphate (the same employed in Feulgen test). Finally they are stained for a short time with Delafield hematoxylin to obtain a contrast stain. The glycogen after this procedure is intensely stained a carmine red.

To distinguish the glycogen from some other polysaccarids that can also be stained with the glycogen tests I have employed some control slides which were placed in a solution of filtered saliva in distilled water for an hour at 37° C. This was used to destroy all the glycogen present by the action of glycolitic enzymes.

The study of fat has been done by the technique employed by Goodey (1930). The worms to be tested are collected by a capillary pipette into a small drop of water in a glass capsule. They are then fixed in hot 10 per cent alcohol, containing about 2 per cent glycerin, which is poured over them. A drop of Scharlach R or Nile blue, made up in the appropriate strength, is then added and the capsule set aside to allow the alcohol to evaporate. The worms are finally left in weak glycerin. In the present investigations good results have been obtained using the following solution: Four cg. of Sudan III, 1 cg. of Brilliant-cresil blue and 15 g. of absolute alcohol. The fats present are stained orange color by Sudan III while Brilliant cresil blue gives a contrast stain in blue.

For the study of granulations of doubtful nature some nuclear stains have been employed such as Methyl violet and the Feulgen test for nuclear material which stains the nuclear substance (thymonucleinic acid) a red-violet.

THE FREE-LIVING LARVAE

Busch (1905) tested for the presence of glycogen the larvae of *Ancylostoma duodenale* and concluded that these larvae are free from glycogen. F. K. Payne (1923) in her study of the larvae of *Necator americanus* came to the same conclusion. By daily observations of cultures of *N. americanus, A. caninum* and *Nippostrongylus muris* I found that a small quantity of glycogen can be demonstrated in the lumen of the intestinal tract and also in the body cavity surrounding the intestine of the

rhabditiform larvae of these nematodes during their period of most active feeding. Glycogen is entirely absent, however, in the filariform stages.

The presence of fat in the free-living larvae of parasitic nematodes was carefully studied by F. K. Payne (1922 and 1923). Using Sudan III she was able to demonstrate in larvae of *Necator americanus* large masses of fatty substances stored in the endodermal and mesodermal tissues. She observed too that as the infective larvae migrate the granules disappear and came to the conclusion that energy for migration must be derived from stored nutritive material. Cort (1925), in his paper giving a general summary of the results of investigations on the control of hookworm disease, emphasized the observations of Payne and came to the following conclusions: "The abundance of these granules may be taken as a rough estimate of the physiological age of the larvae. The length of life of a larva, therefore, depends on the rapidity of the using up of these granules, and must in general be measured rather in terms of activity than in time. . . . Under conditions in the soil, in the tropics, where moisture and temperature are favorable the larvae are very active and, therefore, use up their stored food material in a comparatively short time."

I studied the fat present in free-living larvae of *N. americanus, A. caninum, N. muris,* and *Strongyloides fulleborni.* In the young rhabditiform larvae the fat is present in the lumen of the intestinal tract, while in older stages it appears to be stored in the wall of the intestine. The cavity between the larva and its sheath is always free from fat granules. The newly developed filariform larvae are the most rich in stored material which decreases as they get older. Maintaining my cultures for many days I was able to observe a marked reduction of fat granules in the larvae from the older cultures. A more rapid reduction of these stored granules, I have obtained by keeping the larvae isolated in water from the cultures in an incubator at 37° C.

The conclusion of Payne and Cort that the quantity of fat granules is an index of the physiological age of the larvae have been entirely confirmed, but Payne in her paper also expressed the opinion that there are at least two different kinds of granules which are used frequently to supply the requirements of the larvae. Besides the fatty masses she observed smaller granules which were not affected by the Sudan III or Iodine solution. These smaller granules were seen throughout the length of the larvae but only near the periphery. Payne believed that any of these granules which occurred in other portions of the larvae would probably have been obscured by the more abundant and larger stained fat granules. In my observation I have used a contrast stain with Brilliant-cresil blue and have obtained a blue-violet stain of these smaller granules. They are present in large numbers in young rhabditiform stages as well

as in the filariform stages that have lost all their stored fat. They are especially abundant in the peri-eosphageal region where their oval shape and regular organization can be noted. Larvae of *N. americanus* as well as those of *A. caninum* show these granules stained in green after a short immersion of the larvae in a solution of Methyl violet. These granules cannot be considered as containing stored substances but must be regarded as nuclear material. I also studied with different stains these granules in the larvae of *Rhabdias ranae* which on account of their small size can be dried easily on slides. I found in these larvae both with the Methyl violet and the Feulgen nuclear stains a granular nuclear material present in large quantities especially in the peri-esophageal region. Figure I is a rhabditiform larvae of *Rhabdias ranae* after the Feulgen nuclear stain. The nuclear granules (thymonucleinic acid) can be seen especially grouped in the anterior part of the larva.

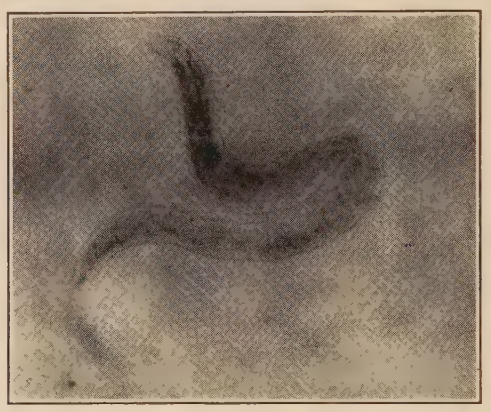

Fig. 1. Rhabditiform larva of *Rhabdias ranae* after Feulgen staining test. Many nuclear granulations are present especially in the anterior part of the larva.

OTHER LARVAE OF PARASITIC NEMATODES

Observation on larvae of *Trichinella spiralis* encysted in the muscles have shown the presence of fat and also of glycogen. I had no opportunity to stain larvae of Filariidae. We have in the literature, however, an observation by Brault and Loeper (1904) that on a slide of microfilariae of *Acanthocheilonema perstans* furnished them by Prof. Brumpt they were not able to see any glycogen.

THE LARVAE IN LUNG MIGRATION

The larvae of *N. americanus, A. caninum, Nippostrongylus muris,* and *Ascaris lumbricoides* were studied during lung migration (figs. 2, 3, and 4). Larvae of *N. americanus* collected from the lungs of mice experimentally infected were stained with iodine solution. The character-

istic brown stain that appears when glycogen is present showed the presence of this substance in the lumen of the intestinal tract of the larvae, and also in the body cavity, especially in the anterior and posterior extremities of the larvae. The glycogen was not present in a typical granular disposition but in masses without a definite outline. The total quantity of glycogen appeared to be very great in contrast with the infective filariform larvae in which glycogen was completely absent.

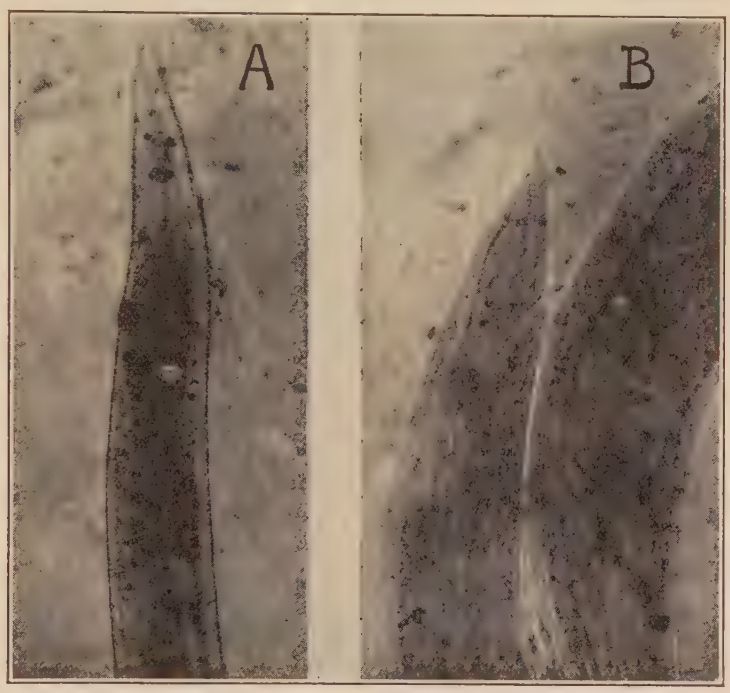

Fig. 2. Larvae of *Ascaris lumbricoides* collected from lungs of mice and stained with fat stain. (A) After 7 days from infection, (B) after 9 days.

Larvae of *N. americanus* collected from the lungs of mice experimentally infected were also stained with Sudan III and Brilliant-cresil blue. Larvae collected two days after infection, when they first appear in the lungs, showed only a small quantity of fat in the body cavity. Larvae collected at a longer time after reaching the lungs show a great quantity of fat present especially in the intestinal tract. It seems natural from these observations to consider that the larvae of *N. americanus* in their lung migration used first the fat stored in the intestinal cavity. Then glycogen is formed by new food taken, and then for a second time new reserves of fat are stored.

Experiments with *A. caninum* and *Ascaris lumbricoides* in mice and with *N. muris* in rats showed the same reaction to the glycogen and fat

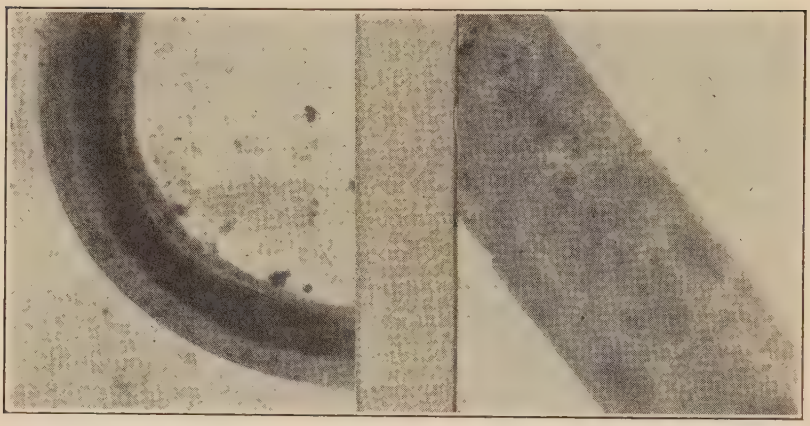

Fig. 3. (Left.) Larvae of *N. muris* from lung of rat after fat stain.
Fig. 4. (Right.) Larvae of *N. americanus* from lung of mouse observed under high power after fat stain. Residual black substances can be seen.

stains in these forms during lung migration. In the larvae of *N. americanus* which appear to have grown somewhat in size black residual substances were observed in the posterior part of the lumen of the intestinal tract (see fig. 4). They are also present in the lumen of the intestinal tracts of *A. caninum* and *N. muris* but have never been seen in the larvae of *A. lumbricoides*. These black granules may possibly be regarded as products of disintegration of blood (hematin), and this hypothesis suggests that there may be a difference in the feeding habits of the larvae of *N. americanus* and *A. caninum* and those of the larvae of *Ascaris lumbricoides*. But these black residuals might also be regarded as particles of coal that are often present in the pulmonary alveoles and constitute the pulmonary anthracosis, a common histological finding in lung sections.

Other authors have noted an increase in size in the lung migration of *A. lumbricoides* as well as *N. americanus* and other nematodes. Schwartz and Alicata (1934) in their paper on the development of *Necator americanus* in guinea-pigs have noted an increase in size of the larvae during the lung migration from 500 to 811 μ in length. Studying their table 2 we can also realize that the greatest difference between the free living stages and the larvae collected from the lungs is not so much in their length as in their maximum width. From 22 μ as free living infective larvae they grow to 57 μ as 5-day-old larvae from the lungs, an increase in width of almost three times. The drawing given by Schwartz and Alicata shows that this increase in width is due to the enormous increase of the intestinal cavity.

My present observation of lung migration of *N. americanus* in experimental infections in mice confirms the observations of Schwartz and

Alicata and gives a tentative explanation of the physiology of these larvae during their lung migration. These nematode larvae, during their lung migration, first use the food material stored in the body cavity and then they feed. This food is utilized as glycogen in further development. Then it is stored as fat in the intestinal tract which is considerably enlarged and later in the body cavity.

THE ADULTS

My observations on adults of *Rhabdias ranae* and *Ancylostoma caninum* agree with the earlier work in showing the presence of glycogen (Weinland, 1901, 1902, 1903). In an accidentally contaminated culture of *A. caninum,* I had also occasion to observe the glycogen stain in a free-living nematode, the species of which was not determined. This free-living adult was found to be full of glycogen and contrasted very much with the filariform larvae of *A. caninum,* present on the same slide, which remained unstained.

The presence of fat in the adults of both parasitic and free-living nematodes has been recently studied by Goodey (1930). The fat is present in the lumen of the intestinal tract but especially in the body cavity, in the peri-esophageal cavity and accumulated in the posterior extremity of the worm.

As was noted by Goodey this fat is disposed in small spherical droplets. In the adult worms the fat can be considered as being present in a greater quantity than in any other stage of the life cycle. In the present

FIG. 5. Tail of adult of *Rhabdias ranae* after fat stain.

research I had occasion to see under the microscope that the droplets of fat had a tendency to flow together and form larger droplets. The disposition that we observe in small distinct droplets can be partially due to a great degree to this tendency of the fat to flow together after the action of fixing fluid (see fig. 5). Making a comparison between the free-living nematodes and the parasitic nematodes we would expect to find fat more abundant in the free-living nematodes where the storage of food has a much greater importance. Studying the different degrees of parasitism we observe that fat is more abundant in the nematodes that do not feed on the host's tissues. The study of the distribution of fat in adults of *A. caninum* has shown (Giovannola, 1935) that the fat is especially stored in rays of the male bursa, and the quantity of fat present there is comparatively greater than in any other part of the male worm or the female worm. The rays have been considered as organs for reserve of food and these reserves present in males and absent in females have been explained by considering that during the long periods of copulation the females remain attached to the intestinal mucosa and can feed while the males are attached to the females but generally detached from the host mucosa. This is an example of the increase of reserves of food during a period when the parasite was not feeding from the host.

GERMINAL ELEMENTS AND EGGS

While the fat is found most abundantly in the adult stages the glycogen is present in greater quantities in the mature germinal elements than in any other stage. This observation was first made by Brault and Loeper (1904), then by Busch (1905) and confirmed in the present research. In cross sections of the adult worms as well as in the systematic studies of development of eggs I observed that the glycogen is present in the largest quantity in the first germinal cell and then decreases little by little as the eggs are fertilized, the shell formed, the eggs divided, and the embryos formed.

The eggs of ascaris studied in cross section of the uterus and stained with the methods of Best and Bauer showed a marked difference between the young stages which appear intensely stained and the more developed stages in which the stain is less intensive. The eggs of ascaris maintained in culture at 30° C., then treated with a solution of 30 per cent antiformin to destroy the shell and stained with iodine solution or crushed on the slide and successively stained by the methods of Best and Bauer, show a decrease in the quantity of glycogen until it is almost absent in the fully developed embryos. They show too the presence in the unsegmented eggs of a liquid full of glycogen between the protoplasmatic substance and the shell. This liquid is used up in the further stages of development and is absent in the eggs with a fully developed

embryo. In figures 6 A, 6 B, 6 C, and 7 can be seen the decrease of glycogen in different stages of the development of the eggs.

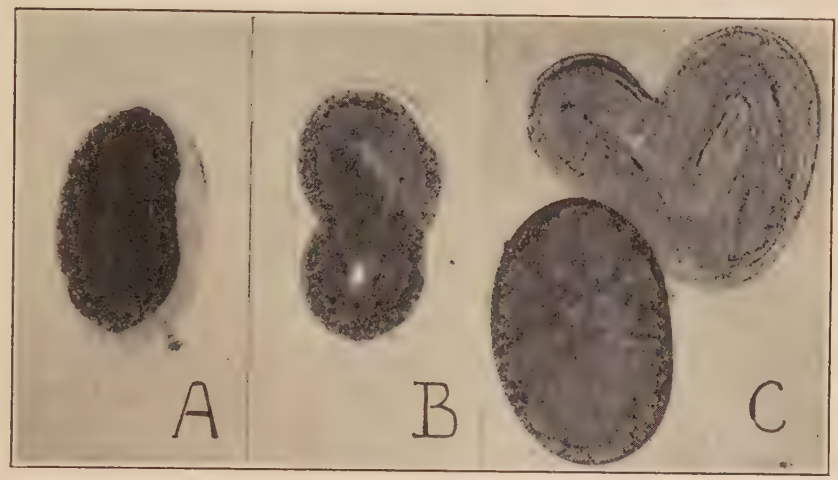

Fig. 6. Three successive stages of development of eggs of *R. ranae* after glycogen stain.

On the other hand the fat that is almost absent in the first stage of the germinal cells appears in the completely matured but not fertilized eggs and then decreases after fertilization. This has been observed by staining cross sections of the uterus and oviduct of *A. lumbricoides* containing eggs in different stages of development. The same observation has been made in the adult females of *Rhabdias ranae* full of eggs in

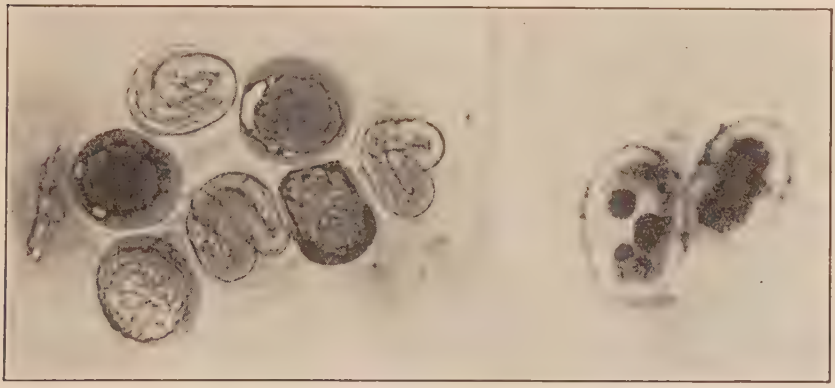

Fig. 7. (Left.) Different stages of eggs of *A. lumbricoides* after glycogen stain. Note the decrease of glycogen in the fully embryonated eggs. The masses not stained in the unembryonated eggs are fat.

Fig. 8. (Right.) Eggs of *Ascaridia galli,* kept for four years in an ice box, after fat staining. One egg is degenerated and shows big fat droplets.

which the eggs not embryonated present a considerable quantity of fat in very small droplets while this is very much reduced in the egg with a fully developed embryo. In the eggs that are not fertilized a fat degeneration can occur. This fat degeneration occurs also in the eggs that are not kept in good condition for further development. This fat degeneration I had occasion to see in the unfertilized eggs of *Ascaris lumbricoides* as well as in some eggs of *Ascaridia galli* kept in an ice box for four years (fig. 8).

DISCUSSION

The glycogen present in the free-living rhabditiform larvae is formed by the food taken during the period of intense nutrition and then used in the rapid growth from the rhabditiform to the filariform stage. A great quantity of glycogen appears in the first stages of parasitic life (lung migration). This substance is used in the rapid growth of parasites during this period. The greatest quantity of glycogen is observed in the mature germinal cells. It then decreases until the embryos are practically without glycogen. *The glycogen is then especially present in all the stages that precede a rapid and intense development. This substance is a reserve of energy.*

The fat began to be stored in the second period of life of the rhabditiform free-living larvae. The quantity is very great in the first filariform stages then decreases and we note another increase of fat during the lung migration when the larvae feed again. This increase is not very rapid because the larvae during this period transform the food taken into glycogen and use it for their growth. The quantity of fat present in the parasitic nematodes reaches its peak in the adult stages. The germinal cells are practically without any fat but when these elements are completely formed the excess of glycogen is transformed into fat and stored. The glycogen present in the unsegmented eggs is all utilized in the formation of the embryos while the fat stored can be found until the embryo is fully developed and represents the substance that will be utilized in the first period of free life. *The fat is then especially present in all stages that precede periods of scarce nutrition or of fasting or in the adults during their sexual life. The fat is, therefore, a reserve of food.* In Diagram 1 is shown the conception gained from this study of the relation of stored glycogen and fat to the different stages of nematode development.

SUMMARY AND CONCLUSION

1. The free-living larvae of *Necator americanus* as well as other parasitic nematodes show a small quantity of glycogen during the period of intense nutrition of their rhabditiform stages.

2. The fat granules stored in the filariform larvae of *Necator americanus* and other parasitic nematodes (*A. caninum, N. muris,* and *S. fülleborni*) have been studied and the conception of Payne and Cort that the quantity of fat granules is an index of physiological age of these larvae has been confirmed.

3. The granules present in free-living larvae that do not stain with fat or glycogen stains have been stained green by Methyl-violet and red by the Feulgen nuclear test. These granules are considered as nuclear substance.

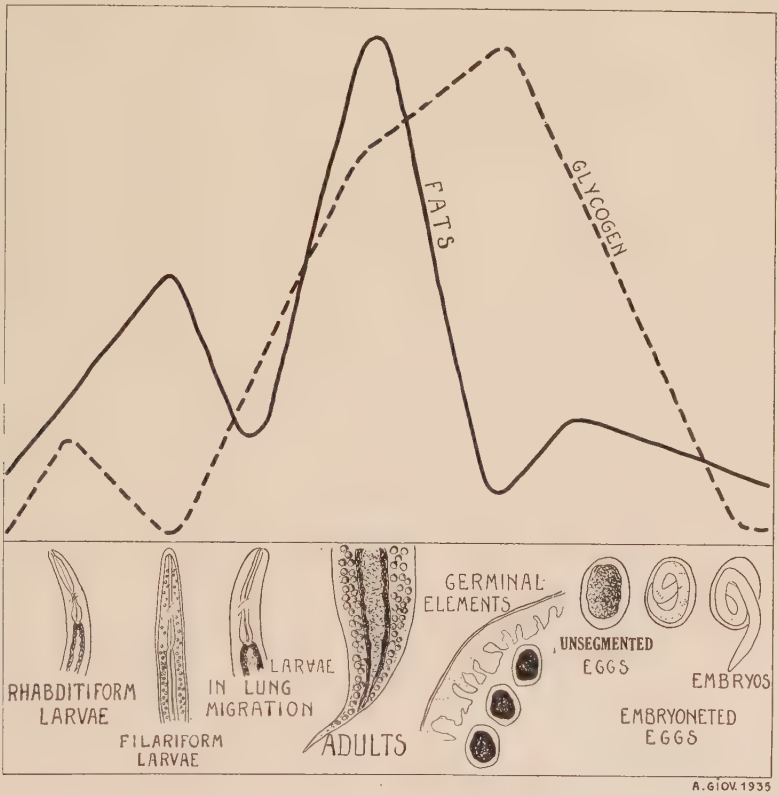

Diagram 1. A schematic representation of the fluctuations in the amount of glycogen and fat present in different stages of nematode development.

4. The larvae of *Ascaris lumbricoides,* as well as the larvae of *Necator americanus, A. caninum,* and *N. muris,* feed during their lung migration and store food. Larvae collected from lungs of mice and rats, experimentally infected, in different periods of time after infection showed a first a rapid increase of glycogen and later of fat in the lumen of the intestinal tract and in the body cavity. During this period the

larvae are morphologically modified, not so much in their length as in their width.

5. In the intestinal cavity of larvae of *N. americanus*, *A. caninum*, and *N. muris* collected from lungs some black residual granules have been observed. These have never been seen in the larvae of *Ascaris lumbricoides* also collected from lungs.

6. Glycogen was seen in the liquid present between the unsegmented protoplasmic mass of the first stages of the eggs and their shell.

7. The substances stored in the nematodes have been distinguished as energy reserves and food reserves. The glycogen is found essentially in those stages that precede a rapid and intense development and has been considered as an energy reserve. The fat is found essentially in those stages that precede a period of less nutrition or of fasting, or in complete sexually developed stages and has been considered a food reserve.

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IN MEMORIAM

Prof. Ettore Marchiafava (1847–1935)

On October 24th, 1935, Prof. Marchiafava died serenely in Rome in his 88th year. He was one of the last survivors of that famous group of early Italian malariologists which included Golgi, Celli, Grassi, Bignami and Bastianelli, and which on the basis of the fundamental discoveries of Laveran and Ross, built the structure of our present knowledge of the species of *Plasmodium* and their mode of transmission by a particular genus of mosquito. To Marchiafava belongs the honor of describing after patient research, the forms and life cycle of *P. falciparum,* noting first the pigment in 1879 before the discovery of the parasite, identifying the ring forms in 1883 and finally (1889) correlating the whole cycle of development of the parasite with the course of Estivo-autumnal fever, just as Golgi had done with *P. vivax* and *P. malariae.* In 1902, with Bignami, he published his great book on "La Infezione Malarica." This was a fundamental work, and a classic in the sense of St. Beuve, being "easily contemporaneous with all time." In its original form it was published first in New York in 1900 in the "Twentieth Century Practice of Medicine." It was called the "best book in English on malaria" and no malariologist can yet afford not to read it.

As a research worker Marchiafava was a methodical and exact observer. His contributions to knowledge were made in spite of inadequate means and equipment. The strange incredulity and even animosity of the medical profession toward the new pronouncements, and the jealousies and bitter polemics which often divided rival malariologists in that golden age of discovery, left Marchiafava untouched. There was an unmistakable modesty, integrity and highmindedness in Marchiafava that in the evening of his life provided him with a multitude of friends and no enemies.

He was first of all a pathologist, becoming full Professor in the University of Rome in 1883, retiring on account of age in 1922. He was the personal, friendly type of teacher, simple, enthusiastic and untiring, but at the same time an acute critic. He remained also a clinician of great prestige and was one of the first to link in his teaching the pathological findings with the clinical picture of disease. He was the personal physician of three Popes and of the Royal House of Savoy, and as he said, followed faithfully the precepts of his master, Guido Bacelli, by trying "to be simple as a straight line, avoiding polypharmacy as he would the devil, and remaining always a sceptic and a philosopher."

He possessed a wide humanistic culture, familiar with the best in English, French and German literature, but especially at home in Latin

and Greek, and he wrote and spoke with charm and clarity, as anyone can see from his brief monograph on "Pernicious Malaria," published in January, 1931, in the American Journal of Hygiene. He was made Senator of Italy in 1913, received in 1926 the Patrick Manson Medal from the Royal Society of Tropical Medicine and Hygiene, and was member of many scientific societies throughout the world. He was active mentally and physically to the end, a frequent visitor to the malaria library of the Malaria Experiment Station in Rome, helpful and unassuming always, as becomes a really great man.—L. W. HACKETT.

RESEARCH NOTES

THE VIABILITY OF *ENDAMOEBA HISTOLYTICA* CYSTS AFTER PASSAGE THROUGH THE COCKROACH

The possibility that cockroaches play a part in the spread of various infectious diseases has been shown experimentally in the case of a number of bacteria, protozoa and helminths. Barber (1912, Philippine Jour. Sci., 7: 521–524) found that cockroaches were not very susceptible to plague bacilli injected into the coelomic cavity. Barber (1914, Philippine Jour. Sci., 9: 1–4) found that cockroaches fed on human cholera feces may harbor the cholera vibrios in their intestines for at least two days. The vibrios did not lose their virulence for guinea pigs after 29 hours in the cockroach intestine. Toda (1923, Jour. of Hyg. 21: 357–361) fed cholera organisms to cockroaches and obtained results similar to those of Barber. Longfellow (1913, Amer. Jour. Pub. Health, 3: 58–61) demonstrated the presence of a large variety of bacteria, including a few pathogenic species, on the legs and in the intestines of common house cockroaches. Diphtheria bacilli were recovered from the legs of cockroaches which had fed on cultures of this organism. In a few cases pneumococci were fed and later recovered from the intestine. Read (1933, Amer. Rev. Tub., 28: 267–272) placed cockroaches in containers with tuberculosis sputum for a period of 48 hours. The alimentary tracts of these cockroaches were then removed. Smears from the intestines were positive for tubercle bacilli in all cases, and inoculation of the digestive tracts into guinea pigs produced typical tuberculous lesions.

Macfie (1922, Ann. Trop. Med. and Parasitol., 16: 441–448) states that the following organisms appear to pass unharmed through the intestine of the cockroach: *Bacillus tuberculosis, B. leprae,* cysts of *Entamoeba histolytica, E. coli, Giardia intestinalis,* and eggs of *Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides, Trichuris, trichiura, Taenia saginata* and *Schistosoma haematobium.* Tejera (1926, C. R. Soc. Biol., 95: 1382–1384) found live cysts of *E. coli* in cockroaches caught in a latrine, and cysts resembling those of *E. histolytica* in the excrement of cockroaches caught in a kitchen near a closet drain. Two young cats were fed the droppings of these cockroaches and both animals developed amoebic dysentery. Pessoa (1927, Rev. Biologia e. Hyg. São Paulo, 1: 90–92. Abst., 1927, Trop. Dis. Bull., 24: 871) fed cysts of *Giardia intestinalis* to cockroaches. Living cysts were abundant in the feces and regurgitations 48 hours after feeding, and a few cysts could be found in the feces after 7 days.

Our experiments were performed with the large cockroach, *Periplaneta americana.* The cockroaches were caught and placed individually in 250 cc. wide-mouth bottles covered by several layers of cheese cloth. A few drops of sterile distilled water were placed in the bottom of each bottle to keep the droppings moist. The cockroaches were starved for two days and the droppings from each bottle were examined for cysts, with negative results.

In order to allow the cockroaches to feed without contaminating the legs and body each insect was placed in a clean glass beaker. Culture cysts of *E. histolytica* with rice flour and a little sugar were mixed in a small glass dish (Lamotte hydrogen-ion indicator dish), 1 cm. in diameter and 0.5 cm. deep, and placed in the beaker with the cockroach. Each cockroach was watched until all the material had been ingested. This was usually accomplished in a few minutes. Eighty-five cockroaches ingested the material. Those which did not eat were discarded or starved for a longer period. After feeding, each cockroach was placed in a clean bottle for observation. Cysts of *E. histolytica* were found in the gut or droppings of all the cockroaches fed.

Our first observations were made to determine the time necessary for the cysts to pass through the intestine. Cockroaches which had been fed were killed at various time intervals and the intestine examined to determine the location of the cysts. The cysts remained for some time in the crop. At the end of 4 to 8 hours

most of the cysts were found in the anterior and middle portion of the intestine and after 16 hours cysts were found in the hind-gut. In from 16 to 20 hours cysts began to appear in the droppings. If the cockroaches were fed again a few hours after the cysts were ingested the material passed more rapidly through the intestine, and cysts appeared in the droppings after 10 to 12 hours. If no second feeding was given the cockroaches usually ceased to pass droppings after about 48 hours. Cysts were found in the hind-gut in a number of cases 72 hours after feeding. When eosin was applied to the cysts either from the droppings or from the intestine after 48 hours it was found that a large number did not take the stain. Some of the cysts in the gut after 72 hours also did not take the eosin stain.

In order to determine whether the cysts which did not take the stain were capable of excystation, they were placed in egg-horse-serum-Ringer medium with rice flour. It was found that cysts taken from the crop 8 hours after ingestion produced good growth in culture in 24 hours. When the gut was removed after 10 to 12 hours and the cysts planted, growth was obtained in from 24 to 48 hours. One culture, started from cysts which had been in the cockroach intestine for 48 hours, was carried through 42 transplants and differed in no way from the culture from which the cysts were originally obtained. We also obtained growth from cysts collected in the droppings at the end of 48 hours. The scant droppings after 72 hours produced no growth, but may not have contained any cysts. No cultures were made from the intestines after 72 hours.

From these observations it seems quite possible that cockroaches which have access to human feces containing cysts of *E. histolytica,* may under certain conditions contaminate food with their droppings, and thus act as passive transmitting agents of this organism.—William W. Frye and Henry E. Meleney, *Department of Preventive Medicine and Public Health, Vanderbilt University School of Medicine, Nashville, Tennessee.*

NOTE ON THE EGG PRODUCING CAPACITY OF *COOPERIA CURTICEI,* A NEMATODE PARASITIC IN SHEEP

The egg production of single species of parasites in experimental infestations in sheep may be calculated more or less accurately on the basis of the number of egg-producing females found post mortem and the number of eggs these worms have produced daily during the period of infestation. From such a series of observations the number of eggs laid in a 24-hour period by one female of the species *Cooperia curticei* has been calculated.

During a period of 126 days, 3 lambs, nos. 61, 63, and 65, received daily doses of infective larvae of *Cooperia curticei,* totaling for the entire period, 63,000, 105,000, and 65,750 larvae, respectively. On post mortem examination, the 3 lambs had, respectively, 4,137, 3,832, and 286 female *Cooperia curticei.* The presence of only 286 female *Cooperia curticei* in lamb 65 was probably due to the fact that, while the total number of infective larvae administered was only slightly less than that administered to lambs 61 and 63, the number of larvae given to the former during the early part of the experiment was but 250 larvae per day, whereas, the number given to lambs 61 and 63 was 500 and 1,000 larvae per day, respectively.

The number of eggs found in one gram of feces was multiplied by the number of grams of feces passed during the 24-hour period represented by the sample, thus giving the number of eggs passed during that particular 24-hour period. All of the values obtained for the 24-hour periods after the egg count had reached the mean value for the entire experiment were averaged and this number was divided by the number of females recovered, thus giving the egg production per female per 24-hour period. The results are shown in the following table.

It is apparent from these figures that there was considerable difference between the number of eggs produced per female in the case of animal 65, harboring only 286 female *Cooperia curticei,* and the number of eggs produced per female in animals 61 and 63, harboring, respectively, 4,137 and 3,832 female *Cooperia curticei.* This result is in line with the findings of other authors to the effect that the egg produc-

	Total *Cooperia curticei*	Total female *C. curticei*	Maximum no. eggs per 24-hour period	Average no. eggs per 24-hour period	Maximum no. eggs per female per 24-hour period	Average no. eggs per female per 24-hour period
Lamb 61 .	8,018	4,137	2,842,400	1,740,810	687	421
Lamb 63 .	6,919	3,832	3,413,600	1,494,009	891	390
Lamb 65 .	455	286	847,200	268,021	2,962	937

tion per female decreases as the total number of parasites increases.—JOHN S. ANDREWS, *Zoological Division, Bureau of Animal Industry, U. S. Department of Agriculture.*

OBSERVATIONS UPON THE MIGRATIONS AND PATHOGENESIS OF *GASTEROPHILUS* LARVAE

The following observations are of interest owing to the fact that it is still uncertain how *Gasterophilus* larvae migrate from the buccal mucous membranes to the stomach (Mönnig, Veterinary Entomology and Helminthology, 1934). The observations in question were made upon a yearling colt which died of tetanus during the first week of December.

Seventeen larvae were located in the lumen of the esophagus (total length 110.0 cm.). One larva, 8.0 mm. in length, was lying unattached in the lumen of the esophagus 46.0 cm. from the stomach. Sixteen larvae which measured from 10.0 to 12.0 mm. in length were firmly attached to the mucous membrane in the posterior portion of the esophagus (the last 17.0 cm.). At the points of attachment well-defined pits were present; these were similar to such lesions which are frequently present where *Gasterophilus* larvae are attached to the cardiac portion of the stomach wall.

The esophageal mucous membrane was dissected away from the underlying tissues; no trace of migrating larvae or their tracts were visible. The submucosa of the tongue and mouth also failed to reveal the presence of any parasites.

The stomach contained two hundred and ninety-nine larvae which varied in length from 14.0 to 18.0 mm. About equal numbers of these were attached to the cardiac and glandular portions of the organ and many more were free in the lumen. When the gastric mucosa was stripped away from the submucosa the latter was observed to be markedly edematous; this was especially the case where larvae were attached in considerable areas to the glandular region; it was less noticeable in the cardiac region. The edema was manifested by the presence of a yellowish, gelatinous material.

A number of the larger larvae in the stomach were identified as *Gasterophilus intestinalis.*

To summarize it is felt worthwhile to record the presence of the larvae of *Gasterophilus* in the lumen of the esophagus, their absence in submucous and peri-esophageal tissues, and the occurrence of a marked edema in the gastric submucosa.—D. E. FAULKNER AND A. A. KINGSCOTE, *Ontario Veterinary College, Guelph, Ont.*

EFFECTS OF INCREASING THE VISCOSITY OF CULTURE MEDIA ON THE MULTIPLICATION OF *TRICHOMONAS HOMINIS*

Among the methods we have employed to change the character of the medium in the lumen of the intestine of experimental animals so as to test the effects of changes in the environment on the growth and multiplication of intestinal protozoa is that of adding to the diet substances such as agar-agar, psyllium seed and gum arabic in an attempt to render the medium more viscous. In a recent paper (Hegner and Eskridge, Amer. Jour. Hyg., 21: 135–150, 1935) we presented the results of

feeding these substances to rats on the number of trichomonads. These indicate that an increase in the viscosity of the medium brings about a decrease in the number of these flagellates. Thus the average number of trichomonads per unit in rats fed on psyllium seed was 55; on agar-agar, 285; and on gum arabic, 7; whereas the number per unit in rats fed on a "normal" diet was 392. Gabaldon (Jour. Parasit., 21: 454–455, 1935) found that the number of balantidia in rats infected with ciliates from a chimpanzee was also less when a diet was used that increased the viscosity of the intestinal contents. His data are as follows: balantidia per centigram in normal rats, 347.4, in rats fed on agar-agar, 60.4; in normal rats, 314.4, on linseed, 92.7; in normal rats, 48.4, on psyllium seed, 34.2.

Since this work was done we have tested the effects of agar-agar on *Trichomonas hominis* in culture, using a pure line of *T. hominis* and a culture medium consisting of Ringer's solution containing 0.5 per cent of Loeffler's dehydrated blood serum. Two experiments were carried out at an interval of 8 days. In the first, four sets of 4 tubes each containing 10 cc. of medium plus agar-agar as indicated, were inoculated each with 2,000 *T. hominis,* incubated at 37° C. and examined on the second, fourth and sixth days with the following results:

Percentage of agar-agar and number of trichomonads per cmm. in experiment 1.

Agar	0	0.1%	0.2%	0.3%
2nd day	162	122	33	0.5
4th day	614	654	114	97
6th day	289	100	25	17
Total	1065	876	172	114.5

In the second experiment 4 sets of 10 tubes were similarly made up and inoculated each with 1,600 *T. hominis* with the following results:

Percentage of agar-agar and number of trichomonads per cmm. in experiment 2.

Agar	0	0.1%	0.2%	0.3%
2nd day	331.8	152.0	154.1	22.2
4th day	56.4	99.7	127.8	83.5
6th day	66.6	32.3	21.5	34.9
Total	454.8	284.0	303.4	140.6

In general the data indicate that *in vitro* as well as *in vivo* an increase in the viscosity of the medium results in a decrease in the rate of multiplication of *T. hominis.* Several factors may be responsible for this result; these will be discussed later in another contribution.—Robert Hegner and Lydia Eskridge, *School of Hygiene and Public Health, Johns Hopkins University.*

ON THE OCCURRENCE OF A SPECIES OF *ANCYLOSTOMA* CLOSELY RELATED TO *A. DUODENALE* IN AN AFRICAN LEMUR

Lemurs or, as they are popularly called, night apes, are not uncommon in certain districts of the Katanga (Belgian Congo). Subsisting mainly upon fruit and a few insects, they live a purely arboreal life and so are very difficult to catch. While I was in the Katanga in March, 1933, I was fortunate to secure four specimens of *Galago crassicaudatus* for helminthological examination. Two of these carried infections with *Subulura otolicni* (van Beneden, 1890); one animal harbored six specimens of a species of *Physaloptera* indistinguishable from *Physaloptera clausa,* (Rudolphi, 1819) recorded previously only from Insectivora (shrews). In the duodenum of another specimen I found two males of a species of *Ancylostoma* whose principal measurements in mm. are tabulated below—

Length	7.750
Width	.400
Distance from ant. extr. to excret. pore	.542

Distance from ant. extr. to cerv. pap.	.530
Oesophagus (length)	.900
Buccal capsule (length)	.150
Spicules	1.400
Distance between cutic. striations	0.0062

On comparing specimens of *A. duodenale* of human origin with those from the lemur, the only differences that I can detect are that, in frontal view of the mouth, the third or posterior pair of teeth are slightly larger than in *A. duodenale* and the copulatory spicules are shorter (1.4 mm. contrasted with 1.9–2.0 mm.). I hesitate

FIG. 1. Anterior end of male specimen of *Ancylostoma duodenale galogoi* from the lemur, *Galoga crassicaudatus.*

to attribute specific significance to these slight differences but, in view of the fact that the host lives an entirely arboreal life in a region where *A. duodenale* is unknown in man, it is difficult to conceive of our parasites being specifically identical with *A. duodenale.* I consequently propose to refer our specimens to a new subspecies, *Ancylostoma duodenale galagoi.* The types are deposited in the Helminthological Collection of the Prince Leopold Inst. of Trop. Med., Antwerp, Belgium. This is apparently the first record of a species of *Ancylostoma* from a lemur. It is interesting to note that worms identified as *A. duodenale* have been recorded from several hosts other than man as follows:

Host	*References*
Pig	O'Connor, 1921. Med. Journ. Australia, Oct. 2, p. 345.
	Legg and Rheuben, 1921. *Ibid.,* Oct. 5, p. 398.
Tiger (*Felis tigris*)	Lane, 1917. Indian Journ. Med. Res., 5: 210.
	Baylis and Daubney, 1922. Mem. Ind. Mus., 7: 335.
	Baylis and Daubney, 1923. Rec. Ind. Mus., 25: 572.
Lion (*Felis leo*)	Schwartz, 1927. Jour. Parasit., 14: 59. (In captivity, Washington.)
Fishing cat (*F. viverrina*)	Baylis and Daubney, 1922. *Ic.*
Gorilla gorilla	Looss, 1911.
Viverra civetta	Adler, *l.c.,* p. 294.
Fennec fox (*Vulpes zerda*)	Mc Clure, 1932. Zoologica, N. Y., 15: 12. (In captivity, New York.)
Cat	Experimentally only.

Dog	In this host the worm sometimes develops experimentally. Records of its natural occurrence are open to doubt. According to Hall, 1923, Journ. Amer. Vet. Med. Ass., 63: 40. Miyagawa has recorded it in the dog at Tokyo. Thapar, 1929, Trans. Far East Ass. Trop. Med. 7th Congr., India, III, p. 265, speaks of it as occurring in dogs in India.
Pan sp. *Hylobates lar*	These hosts are mentioned by Stiles, Hassall & Nolan, 1929.
Silenus sinicus (experimentally)	"Key-Catalogue of Parasites Reported for Primates," Hyg. Lab. Bull. 152, p. 454.

—Louis van der Berghe, *Institut de Médicine Tropicale Prince Leopold, Antwerp, Belgium.*

ORGAN SPECIFICITY OF THE PARASITE *EIMERIA TENELLA*

For some time it has been recognized that the parasite, *Eimeria tenella,* localizes in the epithelium of the cecal pouches of the chicken and is found sparingly, if at all, in other portions of the digestive tract. The sporozoites of this parasite are liberated in the small intestine but evidently are incapable of development there and pass through the small intestine to the cecal pouches where conditions are favorable for development. In order to simulate small intestinal conditions in the cecal pouches, the distal end of a cecal pouch of fourteen chickens, three to four months old, was surgically united to the small intestine and the intestine abligated just posterior to the union, thus making it necessary for all food to pass directly through one of the cecal pouches as it progressed through the intestine. Chickens so operated upon, when the union was posterior to the yolk-stalk, were inconvenienced but little by the operation; they began eating within a couple of hours and their food consumption and weight remained normal. The translocated ceca of chickens examined twenty-four hours following the operation were found to be filled with food material intestinal in appearance, which bore no resemblance to the contents of the cecum retaining its normal connection to the intestine. A cecum was united to the intestine at approximately six-inch intervals along its length, the most anterior union was made just posterior to the gizzard, the next at the mid-point of the duodenal loop and progressively posterior to that part of the intestine normally lying next to the distal end of the cecal pouches. The operation afforded chickens with two cecal pouches one (surgically united to the intestine) histologically normal but physiologically very abnormal, and one (retaining its normal relation to the intestine) which was histologically and physiologically unaltered.

Following the operation the chickens were given sporulated oocysts of a pure line of *Eimeria tenella* by means of a pipette. The time following the operation when the oocysts were administered was dependent upon the point of union of the cecum with the intestine, *i.e.,* if anterior to the entrance of the bile ducts, two hours following the taking of food; if between the bile ducts and yolk-stalk, twenty-four hours later; and if posterior to this point, they were not infected for four days following the operation.

Following the administration of sporulated oocysts both ceca of chickens operated upon, with one exception, became infected and the characteristic hemorrhage was produced. Furthermore, the infection in the translocated cecum was just as severe as it was in the one retaining its normal relation to the intestine. The chicken in which the cecum was united to the intestine just posterior to the gizzard did not become infected in either cecal pouch. The two chickens, however, in which a cecal pouch was united to the intestine at the mid-point of the duodenal loop became infected in both cecal pouches. In these cases the oocysts were administered

two hours after food was taken by the chickens following the operation, a time too short for a great change in the physiology of the cecum to take place. The first generation merozoites, however, were liberated into a very abnormal habitat but were capable of continuing their development despite the change in physiology.

It was found necessary to infect chickens soon after operations in which the cecum was joined far anterior on the intestine since the chickens did not survive the operation for more than six or seven days, due perhaps to the short length of intestine left available for digestion and absorption. The four chickens in which a cecum was united posterior to the yolk-stalk were not infected until four days following the operation and both the translocated and normal ceca of these chickens became as severely infected as did the ceca of normal chickens the same age receiving the same number of oocysts.

The lower end of the small intestine which after the operation was a "blind pouch" did not become a more suitable habitat for the development of the coccidia than in many normal chickens.

From these studies it is apparent that: (1) The sporozoites of *Eimeria tenella* localized in the epithelium of a cecal pouch through which all intestinal material had passed for a period of four days with all the resulting changes in physiology, and (2) the sporozoites escaped from the oocysts and established themselves in the normal and translocated cecal pouches after passing through only the anterior half of the duodenal loop of the chicken.—C. A. Herrick, *Department of Zoology and of Veterinary Science, University of Wisconsin.*

AN EXTRAORDINARY CASE OF MULTIPLE INFECTION WITH THE SPOROCYSTS OF *LEUCOCHLORIDIUM*

The snail, *Succinea retusa,* serves as the best host for the sporocyst stage of the Harmostome, *Leucochloridium.* Many thousands of such snails gathered on the Erie Marsh (Woodhead, 1936) have been examined for young sporocysts. It is evident that the infection is picked up by the snail during the summer and late fall, thus allowing for the sporocysts to reach maturity in the spring and early summer.

In October, 1932, while making a systematic examination of a large collection of snails of all ages, the procedure outlined below was consistently followed. (a) Removal of the shell without any injury to the snail tissue. (b) Examination was always made under a dissecting microscope and using a strong beam of light. (c) The snail was carefully rotated with needles and examined for visible sporocyst tubes which might occur, first on or in any part of the liver tissue, or second, mixed up with the reproductive tissues. The next step depended on the presence or absence of a visible infection. If present, the sporocyst was dissected free from host tissue, fixed and stored. If no sporocyst was apparent, our routine procedure, maintained over the several weeks required for the examination of every snail in the collection, was to carefully tease apart all of the snail's tissue and look for sporocysts living deep within the tissue and not visible on the surface.

After two weeks of steady examination, many stages of sporocysts were secured, varying in size from one-half millimeter up to sporocysts larger than their snail host. Many double infections were also secured. At the end of a long, hard day, as we were about to discard the remains of a small half-grown snail that had been teased into many fragments and recorded as negative, it was noted that in the liver tissue and about the same color (not white as in the ordinary condition) was a peculiar, club-shaped structure that did not look like liver tissue. Only out of curiosity, the tissue was washed and a reexamination was begun. A very small sporocyst was easily dissected from the liver and in the process the presence of another was made evident. For two hours this kept up and a total of twenty-one small, independent sporocysts were secured. Each sporocyst consisted of one club-shaped sac with visible germ-balls packed in the interior, and numerous, short, nutritive branches. Eight of the twenty-one were more advanced than the others and were prepared as sections. The others were used for toto mounts. The sections are being used in a study of the germ-cell cycle in *Leucochloridium* and are showing

many stages. It is a curious fact that this material fixed late in the afternoon has given many chromosome figures. Research workers having to do with the subject of germ-cell cycle in trematodes or cestodes should pay careful attention to the time of day when the material was fixed. This record in conjunction with the study of sections would determine the rhythm of mitosis, and, possibly, maturation. Much of the material used in the present study of the germ-cell cycle in *Leucochloridium* is barren of chromosome figures due, no doubt, to fixation during other times than the peak of the rhythm. That is why the 21 sporocysts are valuable in the study of the chromosomes.

Whether or not any of the little sporocysts would have reached maturity is a problem. I feel confident that they must be recognized as a new species of sporocysts, whose mature condition is unknown. In structure they are decidedly different from any other of the sporocysts encountered to date. They apparently produce but one brood-sac, with the metacercaria developing very rapidly. It is possible that they may never produce a visible, colored sporocyst, but rely on the entire snail being eaten by some bird. The intensive examination of October, 1932, has not been repeated, and consequently we have not again encountered this little sporocyst. In the many thousands of snails examined macroscopically for mature sporocysts, we have never found a colored sporocyst small enough to check with this form. There is very little chance of its being any other Harmostome than *Leucochloridium*. Cell structure, the tissues, the brood-sac and nutritive branches indicate its being a true *Leucochloridium*. Unfortunately, none of the cercarial germ-balls were advanced enough to give a hint as to the structure of the cercaria or metacercaria.

We also have no knowledge of the fate of such a large number of sporocysts in such a small snail. It may be that such a multiple production of small sporocysts is normal for this species. The small size, coupled with the rapidity of development, may also indicate that the mature brood-sacs never extend into the tentacles as do the colored forms. This latter fact may well account for the failure of McIntosh (1932) to find any visible sporocysts for the adults located at Douglas Lake, Michigan. This does not rule out the possibility of birds receiving their infection from colored sporocysts at some distance from the Douglas Lake area.—Arthur E. Woodhead, *Zoology Department, University of Michigan.*

OCCURRENCE OF INTESTINAL AND NASAL MYIASIS IN OKLAHOMA

A case of human intestinal myiasis due to the larvae of a soldier fly, *Hermetia illucens* L. (Stratiomyidae) was recently reported by Henry E. Meleney and Paul D. Harwood (1935, Amer. Jour. Trop. Med., 15: 45–49), of Vanderbilt University, Tennessee. Their specimens were obtained from a ten-year-old white boy; and they mention that, as far as they know, only one previous case had been reported, that by Morgan (1897, Bull. Louisiana Agr. Exp. Stat., Report of Entom., 2d Ser., No. 48: 151–152) in a man from Arkansas. I was glad to get their paper giving a description of the larva and adult as I was enabled to identify thereby what appears to be the third case ever reported of intestinal myiasis due to larvae of this soldier fly.

Last November Dr. E. A. Johnson, of Hugo, Oklahoma, sent to F. R. Hassler, former assistant State sanitary engineer, a larva preserved in formalin with the following note: "This specimen was passed by a child about 6 years of age. Will you please tell me what it is?" Mr. Hassler brought it to me. This dark brown larva agrees with Meleney and Harwood's description of it as it is markedly flattened dorso-ventrally and has a transverse crease on the ventral aspect of the terminal segment, large prothoracal stigmata and those of the terminal segment in a transverse fissure. Replying to my questionnaire regarding number passed and symptoms that led to the examination Dr. Johnson wrote: "Only one was found and that by accident. Fever 104, abdominal pains, vomiting, diarrhea, loss of appetite. Fever, pains and diarrhea ceased immediately after larvae was passed. Calomel followed by 2 oz. caster oil. No other specimens were found."

In addition two other cases of human myiasis have recently come to my attention in University Hospital, Oklahoma City: A 12-year-old farm girl coughed up in a fit of convulsions 2 larvae and 56 pupae of the stable fly or biting fly, *Stomoxys calcitraus* L. (Muscidae), identified by the single anterior hooklet, stigmata and stigmal field. And, from the nose of a young girl patient were extracted 26 larvae and 13 pupae of the Texas screw-worm fly, *Cochliomyia hominivorax* Coquerel (=*americana* Cush. and Patt. according to Aubertin and Buxton (1934, Ann. Trop. Med. and Parasit., 28: 245–254)) (Calliphorinae), readily identified by the two anterior hooklets, the smooth pharynx of the cephalopharyngeal skeleton, the chitinised tracheae and the anterior and posterior spiracles. They agree with Cushing and Patton's (1933, Ann. Trop. Med. and Parasit., 27: 539–551) illustrations (with but one minor exception, the anterior spiracles have ten openings rather than eleven), and convince me that I am dealing with the true parasite rather than the secondary invader, *Cochliomyia macellaria* F., the common apparently harmless species that has so long been confused with *C. hominivorax* as the screw-worm fly of the New World.

For the benefit of those who are interested in distribution, Oklahoma City is 35° 30′ North latitude. No adults were bred out. These specimens are in the author's collection.—W. P. N. CANAVAN, *Department of Bacteriology, University of Oklahoma School of Medicine.*

A NEW TREMATODE PARASITE, *RENIFER WARDI* N. SP., FROM THE WATERSNAKE, *NATRIX RHOMBIFERA,* FROM COLUMBUS, MISSISSIPPI.

On June 2nd, 1932, Mr. J. W. Ward, Instructor in Zoology, Department of Zoology, Mississippi State College, turned over to the writer six fully mature and two immature living worms recently taken from the mouth cavity and esophagus of the Watersnake, *Natrix rhombifera* (Hallowell), that had been captured from the swamp along the Tombigbee River just south of Columbus, Mississippi. The worms were studied at the time and were identified as belonging to the genus *Renifer* Pratt, 1903, subfamily Reniferinae Pratt, 1903 and family Plagiorchidae Luehe, 1901. The worms were mounted and set aside for further study. They appear to be new to science and are herein described under the proposed name of *Renifer wardi,* the specific name of which is in honor of the collector.

Renifer wardi n. sp.

Specific diagnosis:—Reniferinae: Body elongated oval to lancet-shaped, usually widest in pre-acetabular region; somewhat flattened ventrally but weakly arched dorsally; from 1.39 to 1.55 mm. in length and from 0.44 to 0.52 mm. in width, averaging 1.49 mm. by 0.48 mm. Cuticle moderately thick, from 8 to 12 μ, and thickly beset with thorn-like spines from anterior posteriorly to about level of anterior margins of testes. Oral sucker subterminal, 0.235 mm. in length by 0.206

FIG. 1. *Renifer wardi* n. sp. Camera lucida sketch of type specimen showing the general organization of the body. Dorsal view.

mm. in width. Ventral sucker slightly larger than oral suker, spherical, 0.297 mm. in diameter, located medially in central third of body, with anterior margin averaging approximately 0.65 mm. posterior to the anterior margin of body. Prepharynx present, delicate, short, about 26 μ in length. Pharynx transversely oval, 60 by 88 μ, muscular, surrounded by many rather prominent peri-pharyngeal gland cells. Esophagus thin walled, non-muscular, about 95 μ in length, bifurcating well in front of acetabulum. Ceca simple, diverging laterally around the acetabulum to end slightly in advance of level of posterior margin of ventral sucker. Ovary transversely oval, 0.087 by 0.128 mm., lying close to postero-lateral margin of right or left posterior quadrant of ventral sucker (in four specimens to right of midline and to left in two). Oviduct short, leading into a rather large oötype surrounded by numerous gland-cells, the "shell gland." Laurer's canal present. Seminal receptaculum absent. Uterus descending from oötype on opposite side of body from genital pore, making three or four half loops across that side of the body in descending to near the posterior margin of the worm, then turning ventrally and anteriorly to follow a similar course on the opposite side of the body from the descending limb. Turning at level of ovary it swings to opposite side of body before passing diagonally across ventral sucker to become closely applied to cirrus pouch in passing out to genital pore. Eggs numerous, dark brown in color, operculated, fully embryonated when laid, 18 by 36 μ. Vitellaria laterally placed, disposed into two groups on each side of body, with about 12 or 15 follicles in each group. Anterior groups from level of bifurcation of ceca to level of anterior margin of ventral sucker, lateral to ceca, but overlapping these somewhat in posterior limits. Posterior groups from level of posterior margin of ventral sucker to level of mid-region of testes, entirely posterior to termination of ceca except when body is contracted to the right or left. Individual ducts drain individual groups but two ducts from either side of body form a common duct before reaching ovarian region where two common ducts unite to form a small yolk reservoir. Testes opposite, lateral in position, approximately 60 μ behind level of ovary, slightly irregular in outline (not lobed), elongated longitudinally. Measurements are: 0.107 by 0.162 mm. for right testis and 0.102 by 0.125 mm. for left testis. Vasa efferentia unite as they enter the cirrus pouch. Cirrus pouch slender, slightly club-shaped, 0.5 mm. in length by about 0.07 mm. in width, extending through a sinuous course diagonally across body from genital pore to level slightly posterior to bifurcation of ceca and a little to right of midline but well in front of anterior margin of ventral sucker. Cirrus pouch containing a weakly developed cirrus, a relatively short but convoluted ductus, a rather elongated, club-shaped pars prostatica that carries many weakly defined prostatic cells and an elongated, thin-walled vesicula seminalis. Genital pore left in position, midway between left lateral margin of body and oral sucker, at level of anterior margin of posterior third of sucker, formed as a common opening for both male and female organs. Excretory bladder Y-shaped, stem with many anastomosing lateral outpouchings; cornua extending around acetabulum to its anterior margin. Excretory pore slightly dorsal to posterior terminus of body.

Host: *Natrix rhombifera* (Hallowell).

Habitat: Mouth cavity and esophagus.

Locality: Tombigbee River, Columbus, Mississippi, U. S. A.

Type specimen: Writer's private collection, Zoology Department, University of Georgia, Athens, Georgia, U. S. A.

Renifer wardi shows close affinities to no less than four species of the genus, viz., *Renifer aniarum* Leidy, 1890, *R. acetabularis* Crow, 1913 (Kansas Univ. Sci. Bull., 7: 123–134), *R. natricis* MacCallum, 1921 (Zoopath., 1: 140–204), and *R. texanus* Harwood, 1932 (Proc. U. S. Nat. Mus., 81: 1–31). Reference was made in identifying this species also to recent papers on the Reniferinae by Talbot (1934, Trans. Amer. Micro. Soc., 53: 40–56) and Byrd (1935, Trans. Amer. Micro. Soc., 54: 196–225). It is related more closely to *R. acetabularis* than to the other species showing close affinities, but can be distinguished from all by its smaller

size, both in body form and internal organs. It is separated from its nearest relative by its smaller body, smaller suckers and their ratio one to the other, shorter intestinal ceca and smaller dimensions for most of the internal structures. In regard to the short ceca, *R. wardi* is more like *R. natricis*, since in both forms the ceca fail to extend beyond the caudal margin of the ventral sucker. Here again the major dimensions of the body and internal structures suffice to separate the forms.—Elon E. Byrd, *Zoology Department, University of Georgia.*